UNIVERSIDAD PABLO DE OLAVIDE DE SEVILLA

Departamento de Biología Molecular

e Ingeniería Bioquímica

Evolution of *Carex* section *Rhynchocystis* (Cyperaceae): phylogenetic, biogeographic and taxonomic approaches

TESIS DOCTORAL

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Memoria presentada por la licenciada en Ciencias Biológicas **MÓNICA MÍGUEZ RÍOS** para optar al título de Doctor en Medio Ambiente y Sociedad por la Universidad Pablo de Olavide de Sevilla.

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A LUIS.

A PAULA Y RAQUEL.

A MIS PADRES.

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Abstract

Carex is one of the genera of flowering plants with the highest species richness (ca. 2200). Therefore, this genus is an excellent model to understand the complex evolutionary patterns that generate and maintain biodiversity in angiosperms. This thesis focuses on the study of *Carex* section *Rhynchocystis*, a small group of megaherbs located in a well supported clade of the subgenus *Carex*, along with the *sections Ceratocystis, Rostrales, Spirostachyae* and *Sylvaticae*. The boundaries of the section, its major lineages and phylogenetic relationships among its species were evaluated by sequencing and analyzing four regions of DNA. The phylogeny obtained was used to study the biogeographic patterns by estimating times of divergence and reconstruction of ancestral areas. This first study revealed that section *Rhynchocystis* is a relict lineage of Myocene origin that probably had its ancestral area in the SW of the Mediterranean basin. The geological and climatic changes produced on the Earth from the Miocene have been the main cause of the disjunct distribution of the species within *Carex* section *Rhynchocystis*. Isolation of ancestors in these geographically unrelated areas is probably which led to the formation of new species. Afterwards, molecular data were integrated with morphological data through a biometric study of qualitative and quantitative traits, which allowed to reevaluate the taxonomic treatment of the section in an evolutionary context. As a result of these integrative studies, several cryptic taxa emerged. On the one hand we put in value the species *Carex agastachys*, described by Linnaean son of Central Europe, which had been synonimized with the very morphologically similar *Carex pendula*. On the other hand we describe *Carex leviosa*, a new endemic species of the archipelago of the Azores, and we clarify that *C. pendula* subsp. *myosuroides* is the name that should be applied to the populations of the island of Madeira. This thesis reveals that, although the section *Rhynchocystis* is a group of apparent and relatively

abundant plants, detailed and careful studies of its evolutionary history can reveal novel and unexpected results. It is especially noteworthy that in the 21st century a large species such as *Carex agastachys* has gone unnoticed in a continent so supposedly well studied as Europe, which gives an idea of how far we are still from completing the global catalog of biodiversity.

Resumen

Carex es uno de los géneros de plantas con flores con mayor riqueza de especies (ca. 2200). Por ello, este género megadiverso es un excelente modelo para comprender los complejos patrones evolutivos que generan y mantienen la biodiversidad en las angiospermas. La presente tesis se centra en el estudio de la sección *Rhynchocystis*, un pequeño grupo de megahierbas ubicado en un clado bien apoyado del subgénero *Carex*, junto con las secciones *Ceratocystis*, *Rostrales*, *Spirostachyae* y *Sylvaticae.* En primer lugar se evaluaron los límites de la sección, sus principales linajes y las relaciones filogenéticas entre sus especies mediante la secuenciación y análisis de cuatro regiones de ADN. La filogenia obtenida fue usada para estudiar los patrones biogeográficos mediante la estimación de tiempos de divergencia y la reconstrucción de áreas ancestrales. Este primer estudio reveló que la sección *Rhynchocystis* es un linaje relicto de origen miocénico que probablemente tuvo su área ancestral en el SW de la cuenca Mediterránea. Los cambios geológicos y climáticos producidos en la Tierra desde el Mioceno han sido los principales causantes de la distribución disyunta de las especies que conforman la sección *Rhynchocystis*. El aislamiento de los ancestros en esas áreas geográficamente inconexas es probablemente lo que condujo a la formación de las nuevas especies. Posteriormente, se integraron los datos moleculares obtenidos con datos morfológicos a través de un estudio biométrico de caracteres cualitativos y cuantitativos, lo que nos permitió reevaluar el tratamiento taxonómico de la sección en un contexto evolutivo. Fruto de estos estudios integradores salieron a la luz varios taxones crípticos. Por un lado ponemos en valor la especie *Carex agastachys*, descrita por Linneo hijo de Europa Central, que había sido sinonimizada con la morfológicamente muy similar *Carex pendula.* Por otro lado describimos *Carex leviosa*, una nueva especie endémica del archipiélago de las Azores, y aclaramos que *C. pendula*

subsp. *myosuroides* es el nombre que debe aplicarse a las poblaciones de la isla de Madeira. Esta tesis revela que, pese a que la sección *Rhynchocystis* es un grupo de plantas aparentes y relativamente abundantes, el estudios detallado y cuidadoso de su historia evolutiva pueden revelar resultados novedosos e inesperados. Es especialmente destacable que en pleno siglo XXI una especie de gran tamaño como *Carex agastachys* haya pasado desapercibida en un continente tan supuestamente bien estudiado como Europa, lo que da una idea de cómo de lejos estamos aún de completar el catálogo global de biodiversidad.

Chapter 1. Introduction

INTRODUCTION

Motivation

About 140 million years ago, flowering plants appeared and rapidly eclipsed the diversity of other plants. Currently angiosperms account for c. 90% of all terrestrial plants, many of them of great economic importance due to their use as food, textiles, medicines, construction material, etc. Angiosperm diversification proceeded at a such high rate that some scientists refer to it as a kind of floral "Big Bang" (Soltis et al., 2008). "Abominable mystery" was the expression that Darwin used to refer to the origin, rapid diversification and increase in the dominance of angiosperms on Earth without a clear fossil link marking the transition from other ancient plant species (Seward, 1904). Darwin was deeply intrigued by this “mistery” because it appeared to be against the gradual and slow change proposed in his theory of evolution. Since its origin in the Jurassic and its wide diversification in the Cretaceous there have been numerous changes in planet Earth such as the tectonic drift of continents, dramatic climatic oscillations and an active interaction of angiosperms with other organisms (herbivores, symbionts, pollinators, etc.). It is very likely that the essential role played by insects in pollination is the best explanation for this "abominable mystery" (Saporta, 1873) although an alternative hypothesis proposes that angiosperms simply have high rates of mutation, which generates a significant amount of geno-/phenotypes adapted to different environmental conditions, and promotes their speciation by natural selection (Crepet & Niklas, 2009). Currently there are more than 250000 species of angiosperms divided in about 450 families and some 13000 genera (Vargas, 2014).

The family Cyperaceae Juss. with more than 5500 species occupies the seventh place in the ranking of biodiversity among all angiosperms, and the third within monocots after Orchidaceae Juss. (ca. 26.000 species) and Poaceae Barn. (ca. 11.000 species) (Govaerts

et al., 2017). Members of Cyperaceae have a strongly derived morphology, including greatly reduced reproductive organs, which makes correct determination of species often difficult (Naczi, 2009). The genus *Carex* L. is not only the largest of the Cyperaceae, but also one of the most diverse angiosperm genera with approximately 2000 species (Global Carex Group –GCG– , 2015; Hipp et al., 2016). It has a nearly cosmopolitan distribution and it is present in a broad range of habitats (Govaerts et al., 2007; Judd et al., 2007; GCG, 2015; Hipp et al., 2016), making the genus an ideal system to study evolutionary processes. However, *Carex* presents a difficult taxonomy due to its high species diversity, widespread distribution, and difficult morphology (due to reduced characters and /or recurrent homoplasy) (GCG, 2016). Traditional approaches to the taxonomy of *Carex* have largely relied on the sectional classification (Kükenthal, 1909; Chater, 1980; Egorova, 1999) that are used to help organize very large genera and especially used for specific studies of different aspects and objectives as taxonomic or phylogenetics studies among others (GCG, 2016).

The development of molecular techniques since the end of the XXth century has implied a revolution in the field of systematics, evolutionary biology and biodiversity conservation. Although for some plant groups phylogenies have confirmed traditional classifications based on taxonomy (Vargas, 2014), relationships suggested by molecular phylogenetic hypotheses have frequently revealed that classification based on morphological characters is inappropriate for the delimitation of natural groups of taxa (GCG, 2016). This is especially true in *Carex*, where conflicts between molecular phylogeny and traditional, morphology-based classifications have been evidenced in several sections (Roalson et al., 2001; Hipp et al., 2006; Gebauer et al., 2015; Maguilla et al., 2015; Molina et al., 2015). There is a tendency to base taxonomy exclusively on molecular characters (i.e. DNA-based phylogenies), disregarding the taxonomy based

on morphology (e.g. Figueiredo & Smith, 2015). However, a growing number of revised classifications, which combine morphological with molecular data, suggests that to look for a natural classification that reflects the evolution, characters from different sources (morphological, molecular but also cytogenetic, biochemical, embryological, anatomical ...) should be integrated. This integration appears to be particularly essential to understand and clarify the sectional boundaries of the complicated genus *Carex* (Molina et al., 2015; Maguilla & Escudero, 2016; Benitez-Benitez et al., 2017).

Carex section *Rhynchocystis* is a small group of giant sedges from the Old World that has been poorly studied. The present thesis carries out a detailed study of the systematics and phylogenetic relationships of *Carex* section *Rhynchocystis* based on molecular markers, and revises its taxonomic scheme combining this DNA-based data with morphology.

Taxonomic, molecular and cytogenetic background of *Carex* section *Rhynchocystis*

Taxonomic background

Traditionally, the genus *Carex* has been considered within the tribe Cariceae in subfamily Caricoideae Pax within the Cyperaceae (Muasya et al., 2009). Recent molecular phylogenetic studies have rejected the traditional classification of the Cariceae in five genera because all genera except *Carex* were nested within a paraphyletic *Carex* (Starr & Ford, 2009; Waterway et al., 2009). After the rearrangements by the GCG (2015), a more broadly circumscribed *Carex* became the only genus within the tribe and the subfamily, including all satellite genera (*Cymophyllus* Mack, *Kobresia* Willd, *Schoenoxiphium* Nees, and *Uncinia* Pers). The

study of the genus as a whole has derived in taxonomic rearrangements based on phylogenetic studies (Starr et al., 1999; Roalson et al., 2001; Waterway & Starr, 2007; Waterway et al., 2009; Yen & Olmstead, 2017). Traditionally, *Carex* had been divided into four subgenera based on morphology: *Psyllophora* (Degl.) Peterm., *Vignea* (lestib.f.) Peterm., *Vigneastra* (Tuck.) Kük. and *Carex* (Kükenthal, 1909). Phylogenetic studies were partially incongruent with this subgeneric classification (GCG, 2015, 2016), showing the topological arrangement of the genus into four main clades (Fig. 2): 1- a first clade containing a small group of species from the sections *Siderostictae* Franchet ex Ohwi, *Hemiscaposae* C.B.Clarke, *Hypolytroides* Nelmes, and *Surculosae* Raymond. 2- a second clade including most species of *Carex* subg. *Carex* plus members of *Vigneastra*; 3- a third clade that consists of most *Vignea* taxa; 4- finally, the fourth clade embraces the remaining former genera of the tribe Cariceae (*Schoenoxiphium* *Kobresia*, *Uncinia* and *Cymophyllus*) together with some species from subg. *Psyllophora* .

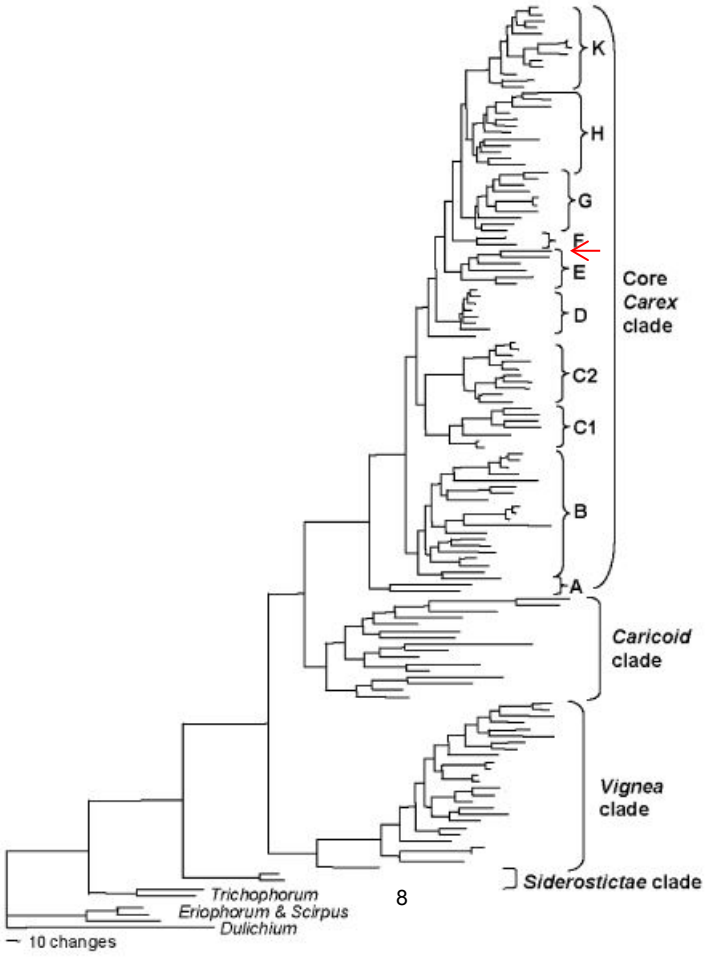

Figure 1. Phylogenetic tree of genus *Carex* extracted from Waterway et al. (2009): analysis of maximum parsimony of the combined regions ITS, ETS, *trn*L and *trn*L-*trn*F of 140 species. The main lineages of the Cariceae tribe are indicated. The name "caricoid clade" is designated for the clade that amalgamates the genera *Kobresia*, *Schoenoxiphium*, *Uncinia* and representatives of the subg. *Psyllophora* of the genus *Carex*. Red arrow indicates section *Rhynchocystis* position.

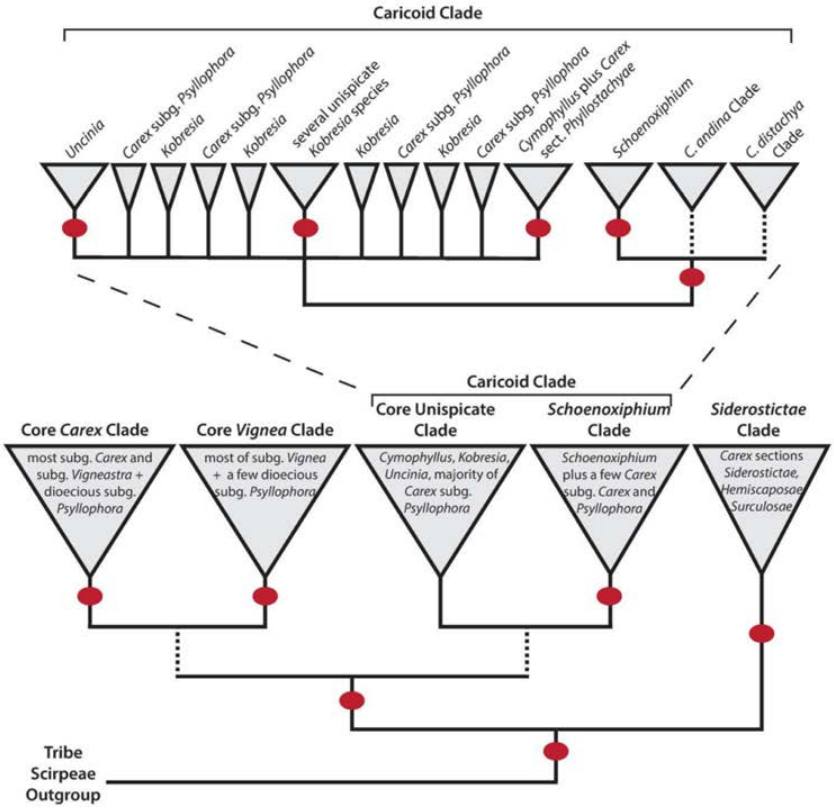

Figure 2. Summarized phylogenetic tree of tribe Cariceae based on molecular phylogenetic studies up to 2015. Solid lines show relationships supported by all or most studies. Dotted branches show frequently, but more inconsistent, relationships among studies. Red circles for clades with consistently high bootstrap support among studies. Figure from GCG (2015)**.**

Carex section *Rhynchocystis* (Dumortier, 1827) belongs to subgenus *Carex*, and is placed in the core *Carex* clade (GCG, 2016; Fig. 1). It is a small section with a disjunct distribution in western Eurasia and North Africa, and in Eastern Tropical and Southern Africa (Fig. 4). *Carex* section *Rhynchocystis* is morphologically well-defined by the following characters: 1-plants densely caespitose with robust rhizomes; 2- leaves folded, more or less rigid, with the abaxial side glaucous; 3- lowermost bract foliaceous and sheathing, as long as or longer than the inflorescence, 4- female spikes large (60 - 100(210) mm), cylindrical, dense; 5- female flowers with 3 stigmas; 6- utricles trigonous, abruptly narrowed into a small tubular beak (0.4 -0.5 mm), truncated and smooth (Chater, 1980; Luceño, 2008). Several taxonomic treatments of *Carex* section *Rhynchocystis* have been proposed, differing in the number of taxa included (5-9 species; Table 1). Kükenthal (1909) recognized 9 species within what he called section *Maximae* Asch. (1864) (=sect. *Rhynchocystis*). Egorova (1999) later reduced the number of species in *Rhynchocystis* to 6. The remaining taxa were transferred to other sections on the light of systematic studies (Dai & Koyama; Reznicek, 2001; Gehrke et al., 2010; GCG, 2016).

Table 1: Taxonomic treatments of *Carex* section *Rhynchocystis*.

GCG (2016)	**Egorova (1999)**	**Kükenthal (1909)**
Section *Rhynchocystis* Dumort.	**Section *Rhynchocystis* Dumort.**	**(=Sect. *Maximae* Asch.)**
C. bequaertii DeWild	*C. bequaertii* DeWild	*C. petitiana* A. Rich
	C. petitiana A. Rich[1]	
C. mossii Nelmes	*C. mossii* Nelmes	*C. mossii* Nelmes
C. microcarpa Moris	*C. microcarpa* Moris	*C. microcarpa* Moris
C. pendula Huds.	*C. pendula* Huds.	*C. pendula* Huds.
C. penduliformis Cherm.	*C. penduliformis* Cherm.	
		C. joorii L.H. Bailey
		C. shortiana Dewey
		C. jaluensis Kamarov

C. maculata Boott
C. vicinalis Boott

Molecular background

Previous phylogenetic studies have shown that *Carex* section *Rhynchocystis* section as defined by Kükenthal (1909) is a heterogeneous group (Waterway et al., 2009) and that the North American and East Asian taxa should be excluded. Relationships of *Carex* section *Rhynchocystis* with close sections are relatively well established on the basis of molecular phylogenies (Waterway & Starr, 2007; Escudero & Luceño, 2009; Waterway et al., 2009). It is included in a well-supported clade together with sections *Sylvaticae* Rouy, *Ceratocystis* Dumort., *Spirostachyae* (Drejer) Bailey and *Rostrales* Meinsh. However, several molecular phylogenies based on different markers produced partially incongruent topologies that mainly affected the relationships between these closely related sections (Waterway & Starr, 2007; Waterway et al., 2009; Martín-Bravo et al., 2013).

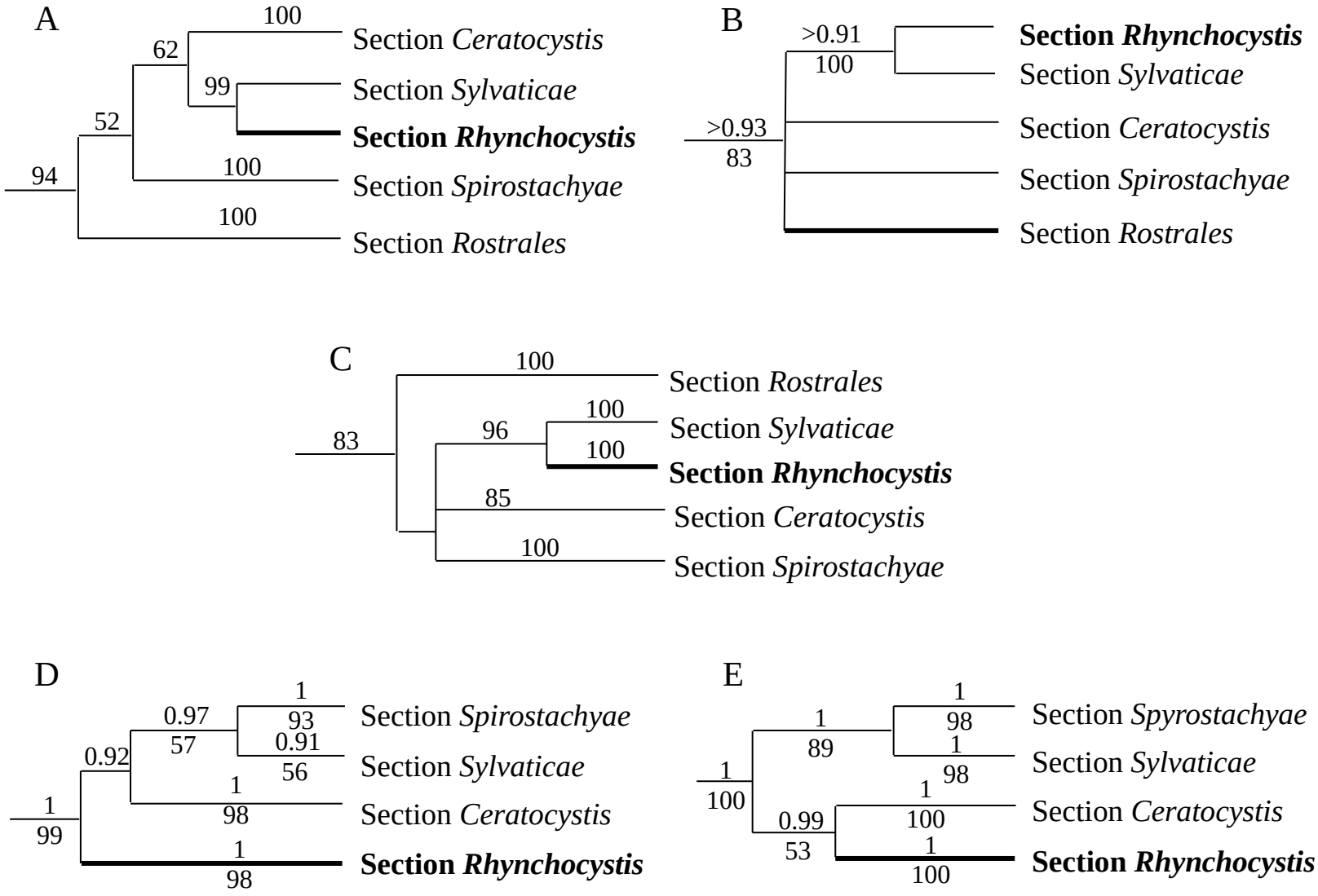
A
100
62
99
52
94
100
100
Section Ceratocystis
Section Sylvaticae
Section Rhynchocystis
Section Spirostachyae
Section Rostrales
B
>0.91
100
>0.93
83
Section Rhynchocystis
Section Sylvaticae
Section Ceratocystis
Section Spirostachyae
Section Rostrales
C
100
83
100
96
100
85
100
Section Rostrales
Section Sylvaticae
Section Rhynchocystis
Section Ceratocystis
Section Spirostachyae
D
1
0.97
93
57
0.91
0.92
56
1
98
1
99
1
98
Section Spirostachyae
Section Sylvaticae
Section Ceratocystis
Section Rhynchocystis
E
1
1
98
89
1
1
98
100
1
0.99
100
53
1
100
Section Spyrostachyae
Section Sylvaticae
Section Ceratocystis
Section Rhynchocystis

Figure 3. Simplified cladograms showing the phylogenetic position of *Carex* sect *Rhynchocystis* in different phylogenetic studies*:* A) Bayesian analysis of the combined nrDNA ITS, ETS-1f, and ptDNA *trn*L–*trn*F regions. Bayesian posterior probabilities are indicated above branches. Modified from Waterway & Starr (2007). B) Parsimony and Bayesian analysis of the combined nrDNA ITS, ETS, and ptDNA *trn*L and *trn*L-*trn*F regions. Bayesian posterior probability and bootstrap values are indicated above and below the branches, respectively. Modified from Waterway et al. (2009). C) Maximum likelihood analysis of the combined nrDNA ETS, ITS, and ptDNA *mat*K regions. aLRT supports (> 75) are indicated above branches. Modified from GCG (2016). D) Bayesian inference analyses based on ptDNA 5′trnK intron marker. Posterior probabilities and bootstrap values are indicated above and below branches, respectively. Modified from Martín-Bravo et al. (2013). E) Bayesian inference analyses of the combined nrDNA ITS-ETS. Posterior probabilities and bootstrap values are indicated above and below branches. Modified from Martín-Bravo et al. (2013). Section *Rhynchocystis* is highlighted in bold font.

As it is shown in figure 3, *Carex* section *Rhynchocystis* is grouped with the section *Sylvaticae* in those cladograms when nuclear and plastidial markers were analysed together (Fig. 3-A, B, C). However, *Carex* section *Rhynchocystis* is sister to the remaining sections if only plastid data is analyzed (Fig. 3-D) whereas sister to section *Ceratocystis* if only nuclear markers are used (Fig. 3-E). The reasons for these different phylogenetic relationships are probably the result of processes such as ancient hybridization / introgression or incomplete lineage sorting.

Cytogenetic background

Carex are non-polyploid angiosperms with unusually diverse chromosome numbers at the genus level, ranging from 2n=12 to 2n=124 (Hipp, 2008; Roalson, 2008).This chromosomal diversity is due in large part to chromosome breakage and fusion, as a result of the behavior of holocentric chromosomes (chromosomes with “diffuse

centromeric activity"; Luceño & Castroviejo, 1991). The rapid rate of chromosome evolution in the genus together with the high species number suggest that chromosome evolution could be considered a primary "cause" of *Carex* diversity (Hipp et al., 2009; Escudero et al., 2014). Known chromosome numbers for species in section *Rhynchocystis* (2n=58 – 62) according with bibliographic data are limited to *C. bequaertii*, *C. microcarpa*, and *C. pendula* (see Table 2).

Table 2: Compilation of chromosome numbers in *Carex* section *Rhynchocystis.*

Species	Diploid number	References
C. bequaertii	58	(Hedberg & Hedberg, 1977)
C. microcarpa	60	(Contandriopoulos, 1962)
C. pendula	58	(Kjellqvist & Löve, 1963)
	58	(Löve & Kjellqvist, 1973)
	58	(Hindakova, 1978)
	58	(Murin, 1978)
	58	(Strid & Franzen, 1981), 1981
	58	(Stoeva, 2000)
	62	(Druskovic, 1982)
	60	(Otonello et al., 1985)
	60	(Druskovic, 1995)

Ecology and distribution of *Carex* section *Rhynchocystis*

Species of *Carex* section *Rhynchocystis* prefer shady conditions where there is a more or less constant water supply as in wetlands, stream edges, and riparian forests (Haines & Lye, 1983; Luceño, 2008). The different species can be found at different altitudes, ranging from sea level in the temperate *C. pendula* (Luceño, 2008) to 3400 m in the tropical *C. bequaertii* (Haines & Lye, 1983). *Carex* section *Rhynchocystis* displays an interesting pattern of disjunction (Fig. 4), with species distributed either in the western Palearctic (temperate Europe, Mediterranean basin and Macaronesia; *C. microcarpa*, *C. pendula*) or in sub-Saharan Africa (*C. bequaertii*, *C. mossii*, *C. penduliformis).* Populations from the Macaronesian archipelagos (Azores and Madeira) have been

considered traditionally either as a variety of the widely distributed Western Palearctic *C. pendula* (var. *myosuroides*), or directly synonymized with it.

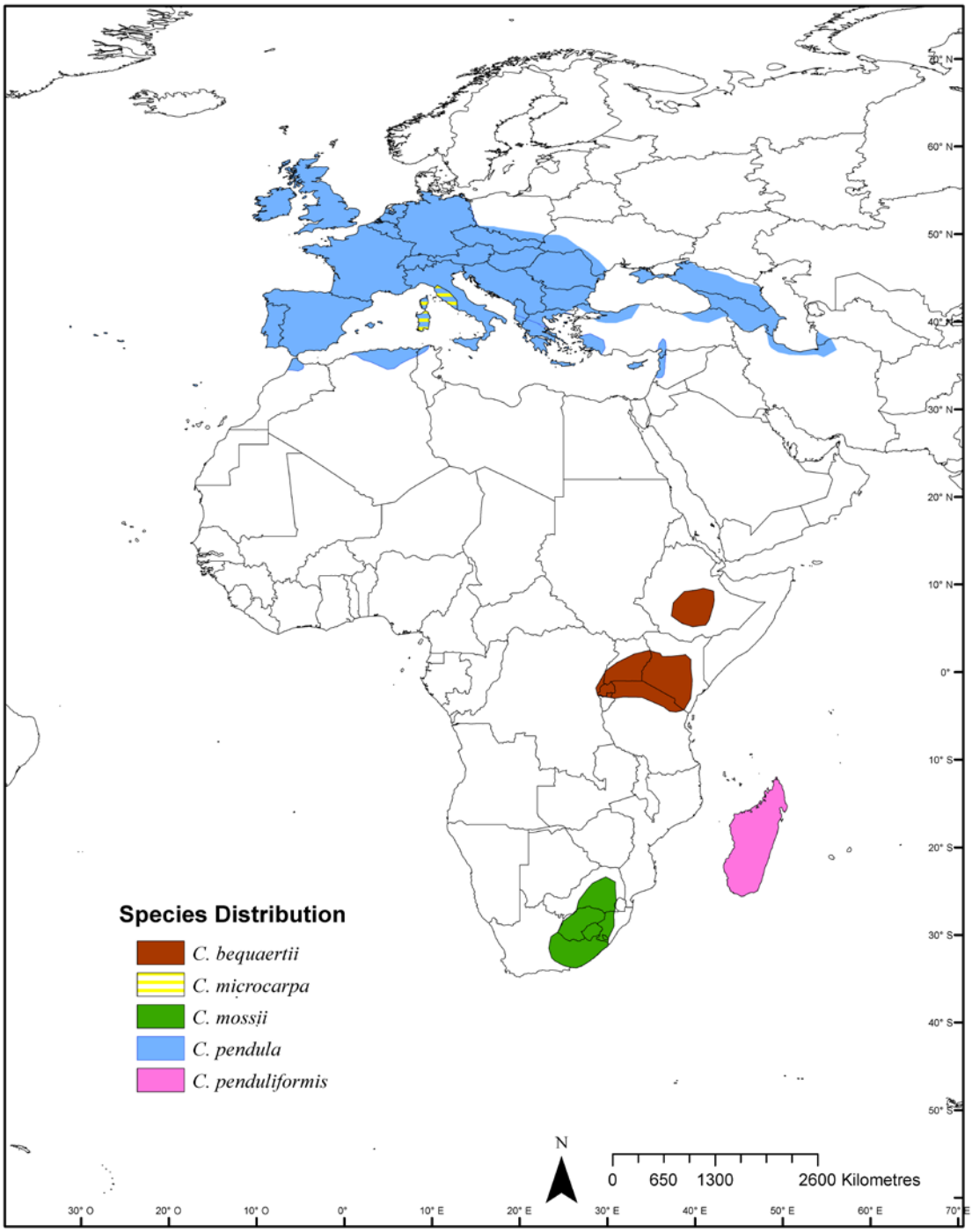

Figure 4. Distribution map of species in *Carex* sect. *Rhynchocystis*. The approximate distribution of the species was obtained from the following floras: Maire, 1957; Schultze-Motel, 1969; Chater, 1980; Meikle, 1985; Nilsson, 1985; Strid & Tan, 1986; Sampaio, 1988; Stace, 1997; Kukkonen, 1998; Egorova, 1999; Zaçac, 2001; Pignatti, 2003; Duhamel, 2004; Jermy et al., 2007; Lauber & Wagner, 2007; Luceño, 2008.

Objectives

The aims of this PhD are: (1) to address the first detailed study of the systematics and phylogenetic relationships of *Carex* section *Rhynchocystis* based on molecular markers, investigating the different processes responsible for the origin and speciation of the different lineages; (2) to study the main biogeographic patterns; and (3) to reevaluate the previous taxonomic classifications of the section based on morphology. The specific objectives of each chapter are described below.

Chapter 2: to infer the phylogenetic, phylogeographical relationships, divergence times and ancestral areas of all species in sect. *Rhynchocystis* based on nuclear and plastid DNA regions.

Chapter 3: to clarify the nomenclature of *Carex pendula* in view of the phylogenetic relationships obtained from the molecular markers.

Chapter 4: to integrate previous DNA-based phylogenetic results with the quantitative and qualitative morphological variation observed, using a rigorous, statistically-based objective framework, and to provide a revised taxonomic treatment for the whole section.

Chapter 5: to elucidate the phylogenetic relationships and taxonomic status of *Carex pendula* populations from Madeira and Azores using DNA analysis combined with a multivariate morphological analysis.

Chapter 6: to compile and discuss all the findings in previous chapters trying to extrapolate, as far as possible, the evolutionary patterns found in the section *Rhynchocystis* to the genus *Carex* in particular and to angiosperms in general.

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Chapter 2. *Carex* sect. *Rhynchocystis* (Cyperaceae): a Miocene subtropical relict in the Western Palaearctic showing a dispersal-derived Rand Flora pattern

ORIGINAL ARTICLE

Carex sect. *Rhynchocystis* (Cyperaceae): a Miocene subtropical relict in the Western Palaearctic showing a dispersal-derived Rand Flora pattern

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ABSTRACT

Aim To evaluate how Cenozoic climate changes shaped the evolution and distribution of *Carex* section *Rhynchocystis*.

Location Western Palaearctic and Afrotropical regions (Rand Flora pattern).

Methods DNA regions ITS, ETS (nuclear), *mat*K and *rpl*32-*trn*L^{UAG} (plastid) were amplified for 86 samples of species from section *Rhynchocystis*. Phylogenetic and phylogeographical relationships were inferred using maximum parsimony, Bayesian inference and coalescent-based species tree approaches. Divergence times and ancestral areas were also inferred.

Results *Carex* section *Rhynchocystis* is a clade that diversified during the middle Miocene in Europe. Most cladogenesis events date to the middle and late Miocene. The Afrotropical group seems to have originated from a colonization event from Europe that occurred in the late Miocene.

Main conclusions Species of the section *Rhynchocystis* in the Western Palaearctic are Miocene relicts. Late Miocene-Pliocene aridification of the Mediterranean rather than the more commonly reported Pleistocene glaciations seems to have shaped the phylogeography of the group. Putative Miocene-Pliocene refugia were probably located in the Mediterranean peninsulas and islands, as well as in the eastern shores of the ancient Paratethys Sea. The colonization of Africa could have been facilitated by Miocene-Pliocene global cooling.

Keywords
ancestral area reconstruction, biogeography, disjunction, estimation of divergence times, Paratethys, Cenozoic relict

INTRODUCTION

In contrast to the number of studies addressing the effects of Pleistocene glaciations on the phylogeography of plants, there are very few studies addressing in detail the effect of Cenozoic climate changes on plant distributions in the Western Palaearctic (e.g. Valtueña *et al.*, 2012; Chen *et al.*, 2014). Climate changes during the Cenozoic were characterized by a transition from the humid and warmer Eocene conditions to the much cooler and xeric climate of the Miocene and Pliocene (Milne & Abbott, 2002). Geodynamic movements occurring in the Western Mediterranean region were responsible for the isolation of the Mediterranean basin from the Atlantic Ocean, causing what has been called the Messinian salinity crisis (5.96–5.33 Ma; Krijgsman *et al.*, 1999). The impact of Mediterranean drying had important effects, not only on the Western Palaearctic but also across the entire Northern Hemisphere (Weijermars, 1988). The effects were also substantial in Africa where the summer monsoon was drastically reduced by the shrinkage of Tethys Sea, causing the Sahara Desert to progressively form during the Tortonian Age (11.6–7.2 Ma) (Zhang *et al.*, 2014) until its definitive establishment 2 or 3 Ma (Kroepelin *et al.*, 2006). These processes altered the composition and distribution of Northern Hemisphere flora. The majority of Cenozoic tropical elements disappeared from most of the Western Palaearctic before the Pleistocene glaciations, remaining in a few southern refugial locations such as the Eastern Mediterranean and Black Sea basins (Milne &

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Abbott, 2002; Médail & Diadema, 2009). It has been argued that only a few taxa persisted in the westernmost part of the Palaearctic, e.g. in the Macaronesian archipelagos (Kondraskov *et al.*, 2015). Nevertheless, there is growing evidence of a Cenozoic origin for species in the Western Mediterranean (e.g. Casimiro-Soriguer *et al.*, 2010; Fernández-Mazuecos *et al.*, 2014, 2016; García-Castaño *et al.*, 2014).

Late Cenozoic climate changes may also have played a role shaping the Rand Flora pattern (Pokorny *et al.*, 2015). The Rand Flora pattern is characterized by the current distributions of related taxa tracing disjunct localities around the margin of continental Africa and adjacent archipelagos (Christ, 1910). Two hypotheses are usually invoked to explain the origin of the Rand Flora: (1) Vicariance: the observed pattern is the remains of a continental flora widely distributed in the past. The original distribution was fragmented due to the increased aridity since the Miocene and restricted to peripheral areas that acted as refugia (Sanmartín *et al.*, 2010; Mairal & Sánchez-Meseguer, 2012). (2) Long-distance dispersal between different parts of Africa (Mairal & Sánchez-Meseguer, 2012; Alarcón *et al.*, 2013).

Carex section *Rhynchocystis* Dumort. (*Rhynchocystis* herein) is a small clade comprising only five species (Table 1). Previous molecular analyses (Global *Carex* Group, 2016) have shown the phylogenetic placement of this section within subgenus *Carex* in a well-supported clade together with sections *Sylvaticae* Rouy, *Ceratocystis* Dumort., *Spirostachyae* (Drejer) Bailey and *Rostrales* Meinsh. *Rhynchocystis* shows a biogeographically interesting range, characterized by a Northern–Southern Hemisphere disjunction coupled with a Rand Flora-like pattern (Fig. 1), with species distributed either in the Western Palaearctic (temperate Europe, Mediterranean basin and Macaronesia; i.e. *C. microcarpa*, *C. pendula*) or in sub-Saharan Africa (*C. bequaertii*, *C. mossii*, *C. penduliformis*). This biogeographical pattern is also displayed by other closely related sections in the genus: *Spirostachyae* (Escudero *et al.*, 2009; Martín-Bravo & Escudero, 2012), *Ceratocystis* (Jiménez-Mejías *et al.*, 2012a) and *Sylvaticae* (Martín-Bravo *et al.*, 2013). The age of the clade can be traced back to the early Miocene, given reports of morphologically similar fossil species from central Europe (*C. limosioides* Negru and *C. plicata* Lańc.-Środ.; reviewed in Jiménez-Mejías *et al.*, 2016).

In this article, we aim to (1) reconstruct the phylogenetic relationships within *Rhynchocystis*; (2) evaluate the possible ancestral areas for the section and its main clades and species; and (3) evaluate the relative contribution of Cenozoic climate changes in shaping the evolution and distribution of the section, with emphasis on the Rand Flora disjunction pattern.

MATERIALS AND METHODS

Sampling

A total of 86 samples (see Table S1 in Appendix S1 in Supporting Information) from *Rhynchocystis* were included in the molecular study: 10 of *C. bequaertii*, five of *C. microcarpa*, 10 of *C. mossii*, 58 of *C. pendula* and three of *C. penduliformis* (Table S1 in Appendix S1). We included two species from each of the closely related sections *Sylvaticae* (*C. sylvatica* Huds., *C. rainbowii* Luceño, Jim.Mejías, M. Escudero & Martín-Bravo), *Spirostachyae* (*C. distans* L., *C. punctata* Gaud.) and *Ceratocystis* (*C. demissa* Hornem., *C. flava* L.), as well as from the more distant section *Phacocystis* (*C. reuteriana* Boiss., *C. trinervis* Dumort.) as outgroup.

A distribution map for *Rhynchocystis* species was created using ArcGIS 10.2 (ESRI, Redlands, California, USA) based on confirmed herbarium vouchers (Míguez *et al.*, unpublished data) and floras (see references in Fig. 1).

DNA extractions, amplification and sequencing

DNA was extracted from silica-dried material collected in the field or herbarium specimens (B, E, M, MA, MHA, MADJ, UPOS, UPS, US, P, PRE, SEV, SS, TUM, Z; abbreviations following Thiers, 2015), using the DNeasy Plant Mini Kit (Qiagen, California, USA). We amplified and sequenced the nuclear ribosomal (nrDNA) internal transcribed spacer (ITS) and external transcribed spacer (ETS) regions. The variability of five plastid regions (ptDNA) was tested (*rpl*32-*trn*L^{UAG}, *mat*K, *ycf*6-*psb*M, *rps*16, *5′trn*K *intron*) in a pilot study on a subset of samples (results not shown). The most variable plastid regions were *mat*K and *rpl*32-*trn*L^{UAG}, which were subsequently sequenced for the complete sampling. These regions have been successfully used to address phylogenetic relationships within *Carex*, including groups closely related to *Rhynchocystis* (Starr *et al.*, 2009; Jiménez-Mejías *et al.*, 2011). PCR conditions and primers followed those described in Global *Carex* Group (2016) for ITS, ETS and *matK*, and Shaw *et al.*, (2007) for *rpl32-trnL*^{UAG}.

Products were cleaned using enzymatic purification ExoSAP-IT (USB Corporation, Ohio, USA) following the manufacturer's protocols and sequenced using Big Dye Terminator v. 2.0 (Applied Biosystems, Little Chalfont, UK) run on an Applied Biosystems Prism Model 3700 automated sequencer. Raw sequences were edited, assembled, aligned and manually corrected using Geneious 6.1.7 (Biomatters, Auckland, New Zealand). IUPAC symbols were used to represent nucleotide ambiguities in ITS and ETS sequences.

Phylogenetic analyses

Five matrices were used for phylogenetic analyses: (1) ITS, (2) ETS, (3) combined nrDNA (ITS and ETS), (4) combined ptDNA (*mat*K and *rpl*32-*trn*L^{UAG}), and (5) combined nrDNA-ptDNA. We performed maximum parsimony (MP) and Bayesian inference (BI) analyses following Maguilla *et al.* (2015). Gene trees were compared prior to concatenation and checked for incongruences of supported nodes with Bayesian posterior probabilities >0.95 and parsimony bootstrap support >75% (Gehrke *et al.*, 2010). For the combined nrDNA and combined ptDNA matrices, we excluded those

Table 1 Summarized taxonomic treatments of *Carex* sect. *Rhynchocystis* at species level. Left column includes the list provided by Global *Carex* Group (2016), which has been followed in this work.

Global *Carex* Group (2016)	Kükenthal (1909)	Egorova (1999)	Natural distribution
Section *Rhynchocystis* Dumort.	Section *Maximae* Asch.	Section *Rhynchocystis* Dumort.	
C. bequaertii De Wild.	*C. petitiana* A. Rich.[1]	*C. bequaertii* De Wild *C. petitiana* A. Rich[1]	E Tropical Africa
C. microcarpa Bertol. ex Moris	*C. microcarpa* Bertol. ex Moris	*C. microcarpa* Bertol. ex Moris	Corsica, Sardinia and C Italy
C. mossii Nelmes		*C. mossii* Nelmes	E South Africa
C. pendula Huds.	*C. pendula* Huds.	*C. pendula* Huds.	Europe, North to 58°N; southwestern Asia; northwestern Africa; Azores and Madeira
C. penduliformis Cherm.			Madagascar
	C. joorii L.H. Bailey[2] *C. shortiana* Dewey[2] *C. jaluensis* Kom.[3] *C. maculata* Boott[3] *C. vicinalis* Boott[3]		

[1]The *C. petitiana* A. Rich type collection contained mixed material of *C. petitiana* as currently delimited (section *Spirostachyae*), and *C. bequaertii* (Nelmes, 1940); following Gehrke's (2010) lectotypification, the name for the species from section *Rhynchocystis* formally became *C. bequaertii*.
[2]The North American *C. joorii* and *C. shortiana* were transferred to sections *Glaucescentes* and *Shortianae*, respectively (Reznicek, 2001), which is supported by recent phylogenies (Global Carex Group, 2016).
[3]The Asian *C. jaluensis* and *C. maculata* have been transferred to section *Anomalae* (Dai & Koyama, 2010); accordingly, the character displayed by *C. vicinalis*, endemic to S India (e.g. bracts sheathless), places this species as allied to section *Anomalae* too, instead of to section *Rhynchocystis*, as already recognized in the lists from Global Carex Group (2016).

samples that lacked one of the DNA regions, whereas for the combined nrDNA-ptDNA matrix, only terminals which lacked more than one DNA region were discarded, resulting in 8% missing data (7.72% missing in ETS, 8.8% in ITS, 8.2% in *mat*K and 7.64% in *rpl*32-*trn*L^{UAG}).

Four matrices were used for a coalescent-based species tree analysis performed using STAR (Liu *et al.*, 2009) as implemented in the STRAW web server (Shaw *et al.*, 2013). Post-burn-in gene trees from the MrBayes analyses of the ETS, ITS, *mat*K and *rpl*32-*trn*L^{UAG} regions were used independently, excluding samples lacking any of the four DNA regions. We failed to amplify some markers for *C. penduliformis* due to poor DNA quality yielded by the sampled herbarium specimens. Due to the lack of ITS sequences for *C. penduliformis,* we performed another species tree analysis excluding the ITS region to retain this taxon in the analysis.

Haplotype and ribotype network

We obtained genealogical relationships among ptDNA haplotypes and nrDNA ribotypes using ptDNA and nrDNA combined matrices, respectively. Statistical parsimony was performed using TCS 1.21 (Clement *et al.*, 2000). The ptDNA matrix was split to analyse *C. pendula* haplotypes independently, following the phylogenetic results, which showed a large genetic divergence between *C. pendula* and the other species (see results). Ribotypes were analysed keeping all samples in the same matrix. Maximum numbers of differences resulting from single substitutions among haplotypes and ribotypes was calculated with 95% confidence limits. Informative indels were coded as a presence/absence character in the matrices.

Divergence-time estimation

We constructed a matrix of 64 sequences: six from *Rhynchocystis* (Appendix S3); 55 from the rest of *Carex*, constituting a representative sampling of the main *Carex* lineages; and three from outgroups, based on Global *Carex* Group (2016). The matrix consisted of 64 combined ETS, ITS and *mat*K sequences with an aligned length of 1696 sites. A dated phylogeny was estimated using BEAST 1.8.0 (Drummond *et al.*, 2012), using an uncorrelated log-normal relaxed clock. Two fossils were used as calibration points, following Jiménez-Mejías *et al.* (2016): *Carex colwellensis* Chandler, 38–33.9 Ma (Priabonian, late Eocene), the oldest reliable known fossil ascribable to *Carex*; and *Carex limosioides*, 23.03–15.97 Ma (early Miocene), the oldest fossil considered to belong to *Rhynchocystis*. Since *C. colwellensis* fossil achene and associated utricle remains (Chandler, 1963; Jiménez-Mejías *et al.*, 2016) display synapomorphies considered unique of modern *Carex*, we placed it at *Carex* crown group (Parham *et al.*, 2012). The fossil *C. limosioides* is considered to belong to *Rhynchocystis*, as it displays the typical combination of carpological characters of this section (Jiménez-Mejías *et al.*, 2016). However, we cannot rule out that these characters have appeared before the crown radiation. Accordingly, to be cautious and avoid an age overestimation for

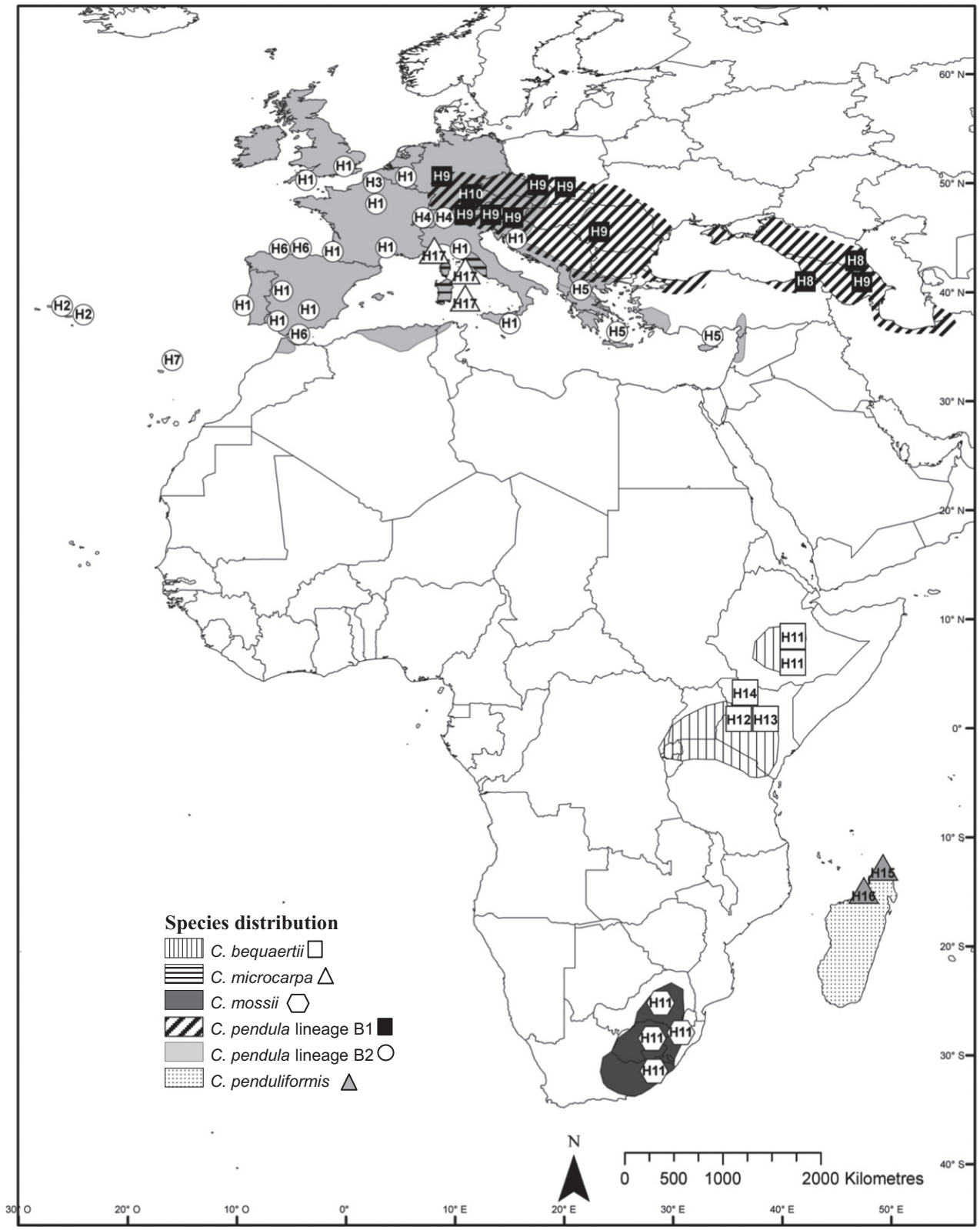

Figure 1 Distribution map of sampled populations/species and haplotypes of the species in *Carex* sect. *Rhynchocystis*. White squares are used to represent *C. bequaertii*; white triangles for *C. microcarpa*; white hexagons for *C. mossii*; black squares and white circles for *C. pendula* lineage B1 and *C. pendula* lineage B2, respectively; and grey triangles for *C. penduliformis*. The approximate distribution of the species was obtained from confirmed herbarium vouchers (Míguez *et al.*, unpublished data) and floras (Maire, 1957; Schultze-Motel, 1969; Chater, 1980; Meikle, 1985; Nilsson, 1985; Strid & Tan, 1986; Sampaio, 1988; Stace, 1997; Kukkonen, 1998; Egorova, 1999; Pignatti, 2003; Duhamel, 2004; Jermy *et al.*, 2007; Lauber & Wagner, 2007; Luceño, 2008; Jímenez-Mejías & Luceño, 2011; Zając & Zając, 2001).

our study group, we placed it at *Rhynchocystis* stem node. Fossil priors were implemented as a log-normal distribution since this distribution is considered the most appropriate for fossil calibrations (Forest, 2009). Fossil records can provide only certainty for the minimum age of a clade, thus minimum age of fossil records is established as the zero offset in our analysis (see Table S3.1 in Appendix S3), as suggested by Heath (2012). Analyses were conducted using two independent Markov chain Monte Carlo (MCMC) runs of 40 million generations each, assuming a birth-death tree prior (see Appendix S3 for details). Run convergence and burn-in were assessed in Tracer 1.5 (Rambaut & Drummond, 2009). A maximum clade credibility (MCC) tree was calculated in TreeAnnotator 1.8.0 (Drummond *et al.*, 2012) using a posterior probability limit of 0.7 and the mean heights option (Appendix S3).

Ancestral area estimation

Biogeographical history of *Rhynchocystis* was reconstructed using dispersal–extinction–cladogenesis (DEC model utilized by Lagrange; Ree *et al.*, 2005), and likelihood implementation similar to DIVA (Ronquist, 1997) using the 'BioGeoBEARS' package (Matzke, 2013, 2014) in R 3.2.5 (R core team 2016), as well as Bayesian binary MCMC Analysis (BBMA) as implemented in rasp 3.2 (Yu *et al.*, 2015). In the BBMA analysis, we used 1000 randomly chosen trees from the posterior distribution of the dated tree estimated using beast 1.7.5 (see above), in order to assess uncertainty in biogeographical reconstructions due to both topological and temporal uncertainty (see Appendix S3). The distribution of *Rhynchocystis* was coded based on the different climatic and biogeographical regions in the Palaearctic and Africa (Fig. 2, Appendix S3).

To test whether the inclusion of founder event speciation in the colonization history of the Afrotropics yielded a better model fit, a separate analysis was run only coding regions in sub-Saharan Africa and Madagascar (Appendix S3).

RESULTS

Phylogenetic analyses

A total of 82 newly generated ETS sequences, 83 ITS (73 newly generated in this study plus 10 from GenBank), 85 *mat*K (83 newly generated plus 2 from GenBank) and 84 *rpl*32-*trn*L^{UAG} newly generated sequences were obtained (Table S1 in Appendix S1). *Rhynchocystis* constituted a well-supported clade (100% BS, 1.0 PP; Fig. 3, see also Figs S1–S4 in Appendix S2) irrespective of data matrix and analytical approach. Incongruences were found between phylogenetic reconstructions using different markers, which did not affect species relationships within *Rhynchocystis*, but the phylogenetic arrangement of different samples within species and also the relative position of outgroup (Fig. 3, and Figs S1–S4 in Appendix S2). For the sake of simplicity, we will discuss topological relationships on the basis of the topology recovered from the BI analysis of the nrDNA-ptDNA combined matrix (Fig. 3).

Rhynchocystis was arranged in two major clades (Fig. 3): Clade A (1.0 PP) included the Mediterranean *C. microcarpa* and the tropical African species *C. mossii*, *C. bequaertii* and *C. penduliformis*. *Carex microcarpa* was monophyletic (A1) and sister to the African species with strong support (100% BS, 1.0 PP). The Malagasy endemic *C. penduliformis* (A2; 1.0 PP) was recovered as sister to *C. mossii* and *C. bequaertii* (A3 and A4, respectively; Fig. 3 and Figs S1, S4, S5 and S6 in Appendix S2). The reciprocal monophyly of the latter two species lacked statistical support.

Clade B included all accessions of *C. pendula* (84% BS; 0.99 PP). This clade was formed by two subclades: B1 (78% BS; 1.0 PP) with samples of *C. pendula* from central Europe to the Caucasus and western Iran (Fig. 3); subclade B2 (95% BS; 1.0 PP) included individuals from central and western Europe, the Mediterranean basin (including northwestern Africa and Cyprus) and Macaronesia (Fig. 3). The species trees inferred using STAR (Fig. S6 in Appendix S2) showed the same supported major clades as the MP and BI trees (Fig. 3).

Phylogeographical analysis of ribotypes and haplotypes

When analysing the plastid sequences with statistical parsimony, 17 haplotypes were identified (Fig. 4). We found differences between the patterns of genealogical relationships displayed by plastid haplotypes versus nuclear ribotypes (Fig. S7 in Appendix S2). There was a higher number of ribotypes than haplotypes; the Tyrhenian endemic *C. microcarpa* was linked to the African species in the haplotype network, whereas it appeared more closely related to *C. pendula* in the ribotype network. The species *C. bequaertii* and *C. mossii* shared H11 despite not sharing any ribotypes (Fig. 4 and Fig. S7 in Appendix S2). Haplotypes H1 to H7 belonged to lineage B2 (Figs 3 & 4) and were distributed throughout central and western Europe and the Mediterranean (Fig. 1). Haplotypes H8, H9 and H10, which corresponded to lineage B1 (Figs 3 & 4), were highly divergent with respect to lineage B2 haplotypes (at least 10 mutational connections) and were distributed throughout central Europe to the Caucasus and western Iran (Fig. 1). *Carex pendula* from Azores and Madeira and *C. microcarpa* from Corsica and Sardinia had specific haplotypes (H2 and H7, and H17, respectively; Fig. 4).

The most frequent haplotype in continental tropical Africa was H11. This haplotype was shared between *C. mossii* from South Africa and *C. bequaertii* from Ethiopia (Fig. 1). In Kenya, we found three haplotypes in *C. bequaertii* (H12, H13 and H14). *Carex penduliformis* was represented by two haplotypes (H15 and H16) with a high number of mutational connections, not only with respect to H11 but also between them.

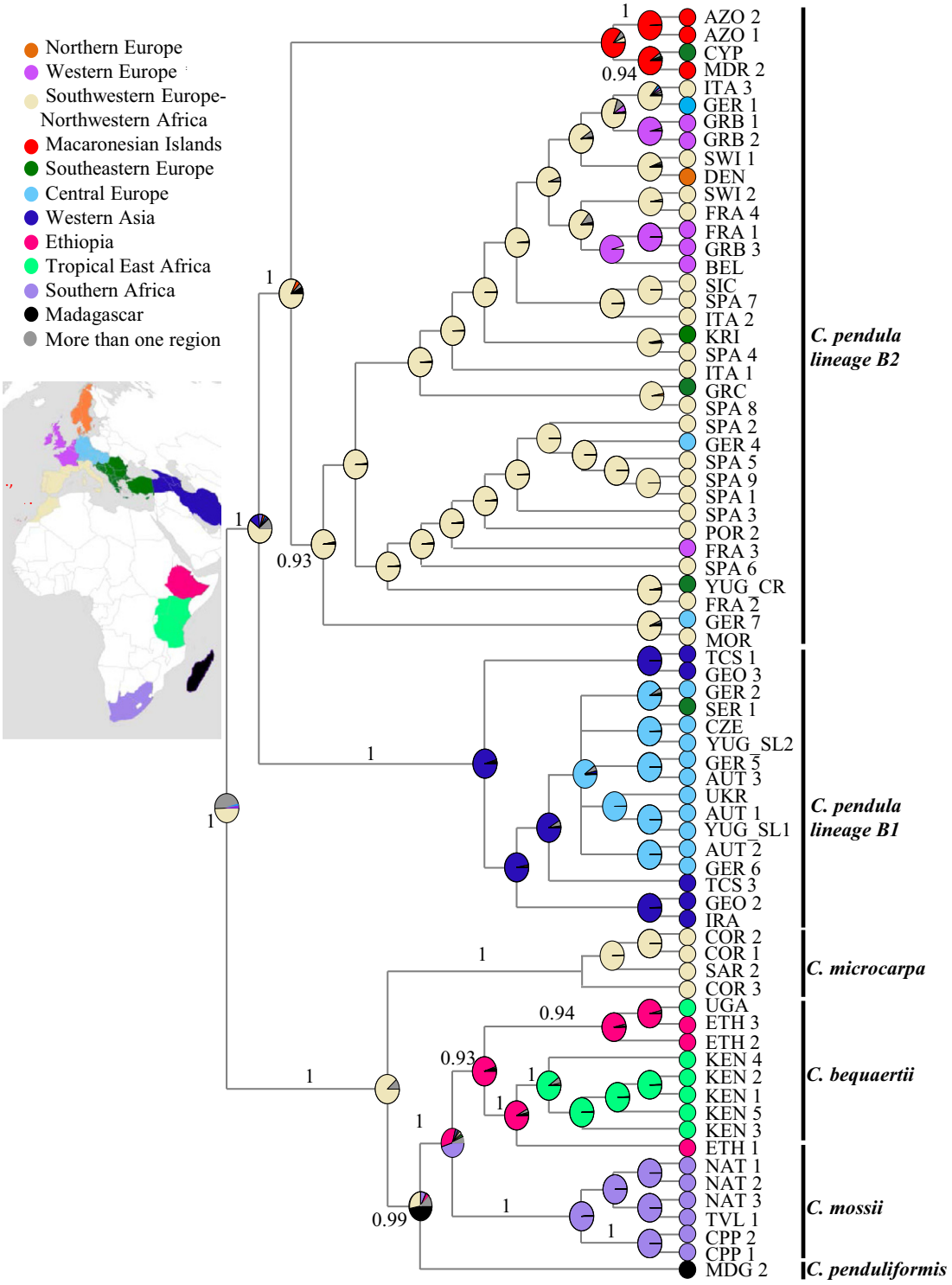

Figure 2 Ancestral area reconstruction inferred using BioGeoBEARS with DIVA-Like+J, coding Palaearctic regions with separate Thyrrhenian Sea islands (see Appendix S3 for details). Tip labels indicate species names and codes of the source regions, following 'botanical countries' as in Brummitt (2001), and including a number when there is more than one sample from the same region as in Appendix S1.

Divergence-time estimation

Divergence of the clade comprising *Rhynchocystis* (stem node) was estimated to have occurred probably during the early Miocene (23.07 Ma, 95% highest posterior density, HPD, interval 28.6–18.36 Ma; Fig. 5, Table 2, Fig. S8 in Appendix S3). Diversification of the section (crown node) was estimated to have begun 16.32 Ma (95% HPD 17.21–

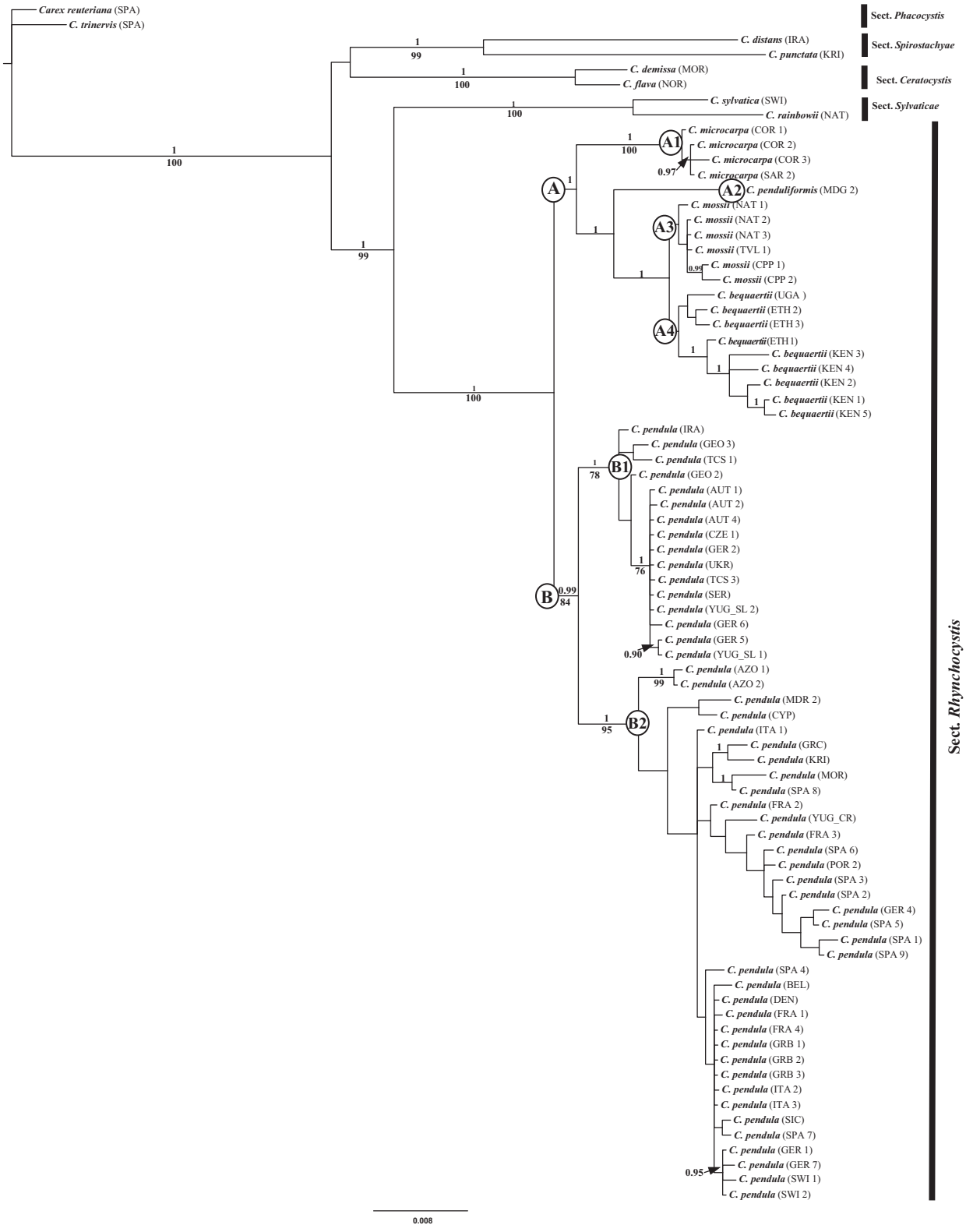

Figure 3 Majority-rule consensus tree of *Carex* sect. *Rhynchocystis* inferred under Bayesian inference using the combined nrDNA-ptDNA matrix (ETS, ITS, *mat*K and *rpl*32-*trn*L^{UAG} regions). Numbers above and below the branches indicate clade support values: maximum parsimony bootstrap and Bayesian posterior probability, respectively. Tip labels indicate species names and codes of the source regions (in parenthesis), following 'botanical countries' as in Brummitt (2001), and including a number when there is more than one sample from the same region. Scale bar indicates substitutions per site.

15.97 Ma). Differentiation of *C. microcarpa* with respect to the African species (*C. penduliformis*, *C. bequaertii* and *C. mossii*) was estimated to have taken place around 11.54 Ma (95% HPD 15.3–7.22 Ma). Separation between the two subclades of *C. pendula* was dated to 10.14 Ma (95% HPD 15.35–4.43 Ma) (Fig. 5, Table 2; Fig. S8 in Appendix S3).

Ancestral area estimation

The biogeographical reconstruction showed similar results irrespective of the analyses performed (Appendix S3). The area of origin of the most recent common ancestor (mrca) of *Rhynchocystis* (the crown node) and the mrca of the tropical African members of the section and *C. microcarpa* was not reconstructed with certainty although it was most likely in the southern Palaearctic (Fig. 2 and Appendix S3). Dispersal from the Palaearctic to Africa was inferred to have occurred via Madagascar or with a slightly lower likelihood twice independently, i.e. once to Madagascar (*C. penduliformis*) and once either to southern Africa (*C. mossii*) or Ethiopia (*C. bequaertii*). Tropical East Africa was probably colonized by *C. bequaertii* via Ethiopia twice independently (Fig. 2), with one colonization event seeming to have taken place in western Tropical East Africa and another in eastern Tropical East Africa.

All analyses indicated that *C. pendula* first occurred in southwestern Europe/northwestern Africa or was widespread in southern Eurasia from where it dispersed all over Europe after splitting into a western and an eastern lineage (Fig. 2). All scenarios in the BioGeoBEARS analyses showed the highest likelihood for DEC models with founder-effect dispersal (e.g. a daughter species occupies a range outside the distribution range of the ancestor). However, the likelihood scores of DIVA-Like models were very similar to those of the DEC+J (see Table S3.2 and Figs S9–S15 in Appendix S3).

DISCUSSION

Systematics of *Rhynchocystis*

Phylogenetic reconstructions of nrDNA and ptDNA regions (ETS, ITS and *mat*K, *rpl*32-*trn*L^{UAG}, respectively) yielded partially incongruent topologies (Figs S1–S4 in Appendix S2). However, incongruences mostly affected relationships between closely related sections, as has been shown in previous studies (Waterway & Starr, 2007; Waterway *et al.*, 2009; Martín-Bravo *et al.*, 2013) and rarely within species in *Rhynchocystis*. *Rhynchocystis* species are morphologically characterized by leaf blades M-shaped in cross section, usually the widest at least 10 mm wide; culms usually longer than 90 cm; and lateral spikes 60–160(260) mm length, with more than 100 utricles per spike. *Carex pendula* and *C. microcarpa* are strongly supported as monophyletic species in the nrDNA-ptDNA combined analysis (Fig. 3), as might be expected given the undisputed taxonomy of both species (Kükenthal, 1909; Chater, 1980; Egorova, 1999). However, it is remarkable that two well-differentiated geographically distinct lineages (B1, B2; Fig. 3) were detected in *C. pendula*. Interestingly, genetic differentiation between these two *C. pendula* lineages for all studied markers is greater than between other species within lineage A (i.e. *C. mossii* versus *C. bequaertii*, *C. penduliformis* versus *C. mossii*/*C. bequaertii*;

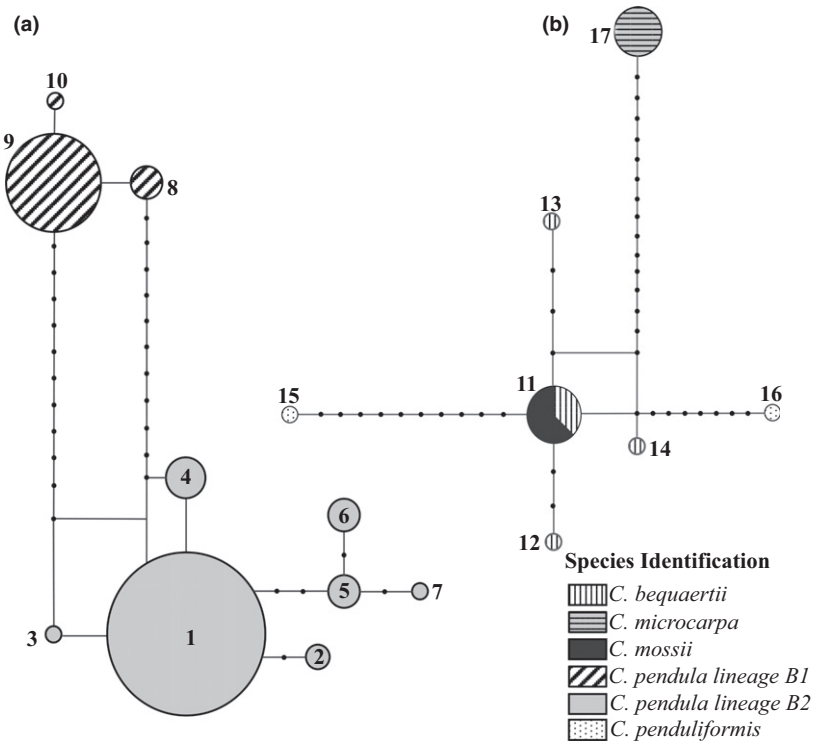

Figure 4 Statistical parsimony network of the 17 haplotypes retrieved from the analysis of the combined ptDNA matrix (*mat*K and *rpl*32-*trn*L^{UAG}). (a) *C. pendula* network. (b) *C. microcarpa*, *C. penduliformis*, *C. mossii* and *C. bequaertii* network. Small black circles represent extinct or unsampled haplotypes, and each line between haplotypes represents a single mutational step. Circle size is proportional to the number of samples displaying the corresponding haplotype. Haplotypes for each sample are given in Appendix S1.

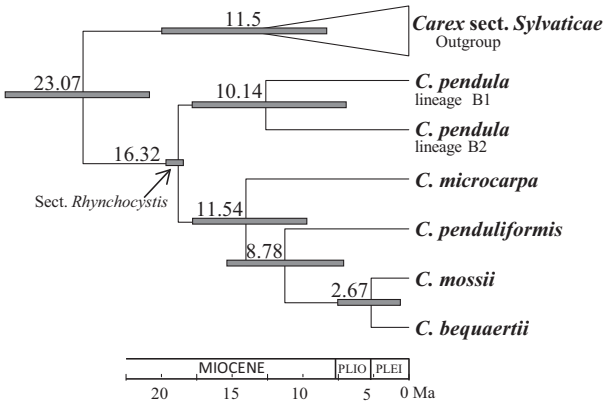

Figure 5 Summary of the maximum clade credibility tree from molecular dating analysis of *Carex* sect. *Rhynchocystis* under an uncorrelated lognormal relaxed-clock model using a matrix of combined ETS, ITS and *mat*K regions. Nodes were collapsed to summarize the main clades (see Fig. S8 in Appendix S5 for the complete tree). Mean ages are shown above the nodes. Node bars represent the 95% highest posterior density (HPD) intervals for the divergence-time estimates of each node with posterior probabilities higher than 0.9. See Table 2 for detailed ages and posterior probabilities inferred for the main clades in section *Rhynchocystis*. PLIO, Pliocene; PLEI, Pleistocene

Table 2 Divergence dates of selected clades obtained in the molecular dating analysis of *Carex* sect. *Rhynchocystis* under an uncorrelated lognormal relaxed-clock model using the combined matrix of ETS, ITS and *mat*K regions in BEAST. Posterior probabilities, mean time to the most common recent ancestor in millions of years (Ma) and 95% highest posterior density (HPD) interval are shown.

Clade	Posterior probability	Mean (Ma)	95% HPD interval Max.	Min.
Carex sect. *Rhynchocystis* (Stem node)	1	23.07	28.6	18.36
Carex sect. *Rhynchocystis* (Crown node)	1	16.32	17.21	15.97
Lineage A: *C. microcarpa* + *C. penduliformis* + *C. mossii* + *C. bequaertii*	1	11.54	15.3	7.22
C. penduliformis + *C. mossii* + *C. bequaertii*	1	8.78	12.89	4.63
C. mossii + *C. bequaertii*	0.99	2.67	5.03	0.62
Lineage B: *C. pendula*	0.99	10.14	15.35	4.43

Fig. 4, Fig. S7 in Appendix S2). The distributions of these *C. pendula* lineages are largely allopatric (Fig. 1). Lineage B1 is distributed in C-SE Europe and SW Asia, in areas that formed the basin of the ancient Paratethys Sea (35–5 Ma). By contrast, sampled populations belonging to lineage B2 are found in the Mediterranean basin, Western Europe and Macaronesia (Azores and Madeira). Molecular phylogenetic data have allowed the detection of different taxa hidden under similar morphologies, even in apparently well-known areas as Europe (e.g. Gurushidze *et al.*, 2008), such as in the closely related *Carex* sect. *Ceratocystis* (Jiménez-Mejías *et al.*, 2012b). Although species circumscription in *C. pendula* has not been under debate, our results could suggest the existence of unnoticed cryptic taxa within *C. pendula*. *Carex penduliformis* is a poorly known species for which only the two type populations from central Madagascar were hitherto known (Chermezon, 1937); we have studied two additional populations from northern Madagascar, which considerably expands the species range. Although Gehrke (2011) indicated the morphological similarity between *C. penduliformis*, *C. bequaertii* and *C. mossii*, there seems to be a high genetic differentiation between *C. penduliformis* and *C. bequaertii*/*C. mossii*, with at least nine mutational steps between their plastid haplotypes (Fig. 4). Interestingly, plastid haplotypes obtained for *C. penduliformis* populations are not only different to *C. bequaertii*/*C. mossii* haplotypes but also one from another (18 mutational steps; Fig. 4). *Carex mossii* and *C. bequaertii* clustered in a strongly supported monophyletic group in the nrDNA-ptDNA combined analysis (Fig. 3) and formed sister groups, the monophyly of which was, however, unsupported (see Figs S1–S4 in Appendix S2). Some populations of *C. mossii* and *C. bequaertii* shared the same, potentially ancestral, haplotype (H11; Fig. 4), which may suggest incomplete lineage sorting (Wendel & Doyle, 1998; Martín-Bravo *et al.*, 2010). Further morphological studies are needed to find clear diagnostic characters and to clarify the taxonomic status of these taxa (Míguez *et al.*, in prep.).

Persistence of *Rhynchocystis* in the Western Palaearctic since the Cenozoic

The persistence of Cenozoic relict taxa in East Asia and North America is a well-documented phenomenon, as the climatic oscillations were limited in these areas in comparison with other regions in the Northern Hemisphere (Milne & Abbott, 2002; Huang *et al.*, 2015). In Europe, many species disappeared during the Pliocene due to the progressive cooling and aridification, followed by Pleistocene glaciations, because both the mostly east–west-oriented mountain ranges and the Mediterranean Sea obstructed many species in their southwards migration to suitable habitats (Milne & Abbott, 2002). Other recent studies are providing growing evidence of Cenozoic relict lineages/species in the Western Mediterranean (e.g. *Erophaca* (Fabaceae), Casimiro-Soriguer *et al.*, 2010; *Naufraga* (Apiaceae) and *Avellara* (Asteraceae), Fernández-Mazuecos *et al.*, 2014, 2016; respectively; *Chamaerops* (Arecaceae), García-Castaño *et al.*, 2014; *Castrilanthemum* (Compositae), Tomasello *et al.*, 2015; among others).

The origin of *Rhynchocystis* dated to the early Miocene (Fig. 5) in southwestern Europe–northwestern Africa (Fig. 2, Appendix S2) is consistent with the continuous fossil record ascribed to *Rhynchocystis* in Europe through the Miocene to the Pleistocene (Jiménez-Mejías *et al.*, 2016). Therefore, the available evidence points to the persistence of *Rhynchocystis* in Europe since the late Cenozoic. The large stature, broad leaves and preference for shady, wet habitats displayed by the

extant species of the section would represent a plausible element of the Lauroid flora which may have covered the continent during this time (Barrón, 2003). Such a scenario would suggest some degree of conservation of the ecological niche in species of *Rhynchocystis* (Wiens *et al.*, 2010).

The only previous calibrated *Carex* phylogeny that included *Rhynchocystis* was provided by Escudero *et al.* (2012), who estimated a considerably more recent origin of the section (Late Miocene-early Pliocene), despite using a much older fossil to calibrate the stem node of *Carex* (*C. tsagajanica* Krassilov; considered highly doubtful by Jiménez-Mejías *et al.*, 2016). In our study, we also included a fossil considered to belong to *Rhynchocystis* (Jiménez-Mejías *et al.*, 2016), although for caution we preferred to place it at the stem node of the section (see Materials and Methods). The contrasting results between these two dating approaches highlight the importance of a careful evaluation of the available fossil record for inference of reliable divergence times and therefore of explicit evolutionary/biogeographical hypotheses (Tripp & McDade, 2014).

Pre-Pleistocene phylogeographical splits in the Western Palaearctic

Speciation in the southwestern Palaearctic (e.g. Mediterranean basin and Caucasus) has frequently been attributed to Pleistocene range shifts (Médail & Diadema, 2009; Martín-Bravo *et al.*, 2010). The Mediterranean peninsulas (Iberia, Italian, Balkan, Anatolia) and islands (Balearic Islands, Corsica, Sardinia, Sicily, Crete, Cyprus) are invoked as important Pleistocene glacial refuges where species requiring warmer climates survived while the ice caps and a tundra-like environment expanded through most of Europe (Médail & Diadema, 2009; Fernández-Mazuecos *et al.*, 2014; Médail & Quezel, 2014). However, in the case of *Rhynchocystis*, most relevant nodes are estimated to be much older (i.e. lineage A versus lineage B, *C. microcarpa* versus African lineage; *C. pendula* lineage B1 versus B2; Fig. 5). In particular, the divergence of eastern and western *C. pendula* lineages during the Middle-Upper Miocene is even older than that of *C. microcarpa* and the African species (Fig. 5).

Since the Oligocene, the Mediterranean basin has been subject to profound climatic changes, initiated with the global cooling trend (Zachos *et al.*, 2001). The closing of the Mediterranean and the Messinian salinity crisis during the late Miocene (Krijgsman *et al.*, 1999) and the establishment of a Mediterranean type climate in the early Pliocene (Suc, 1984) contributed to an aridification process (Zachos *et al.*, 2001) and the spread of steppe-like vegetation (Barrón, 2003). These events led to a dramatic change in the vegetation of southern Europe. Subtropical lauroid vegetation were replaced by Arcto-Tertiary taxa, and the most thermophilous elements largely or entirely disappeared (Ivanov *et al.*, 2011). Such changes would have promoted the retreat of species of *Rhynchocystis* to climatically suitable areas, such as the Tyrrhenian islands for *C. microcarpa*, SW Asia for lineage B1 of *C. pendula*, and possibly the Iberian Peninsula and/or NW Africa for lineage B2 of *C. pendula* (Fig. 1). Such results provide a picture of different parts of the Mediterranean basin acting as refugia during the Cenozoic, which contrasts with the commonly reported predominant role of the Eastern Mediterranean (Milne & Abbott, 2002). Climatic shifts during the Plio-Pleistocene may also have led to range expansions and contractions in *C. pendula*, although there is no clear evidence of ancient secondary contact between the two main lineages. Most likely, the eastern lineage (B1) underwent a severe range restriction to the eastern shores of the Paratethys (present-day southwestern Asia; Fig. 2) during the late Miocene and subsequently recolonized Europe from the East. However, the western lineage of *C. pendula* seems to have sheltered in the warmest moist climates in the Mediterranean basin (Iberian Peninsula, NW Africa), reaching also Macaronesia (apparently by at least two independent dispersals to the Azores and Madeira archipelagos; Fig. 2). It migrated from its refuge areas to central, northern and western Europe during the range expansion–contraction cycles associated with Plio-Pleistocene climatic fluctuations.

A Rand Flora pattern in *Rhynchocystis* originated via long-distance dispersal facilitated by Mio-Pliocene global cooling

Our results suggest a scenario of long-distance dispersal from the Mediterranean to sub-Saharan Africa (Fig. 2 and Appendix S3) as the most likely explanation for the colonization of the Afrotropics. Long-distance dispersal events have been proposed to explain disjunct distributions between the Palaearctic and sub-Saharan Africa in *Carex* (Gehrke & Linder, 2009; Martín-Bravo & Escudero, 2012; Gizaw *et al.*, 2016), *Geranium* L. (Geraniaceae; Fiz *et al.*, 2008), *Lychnis* L. (Caryophyllaceae; Popp *et al.*, 2008) and other plant species (Gehrke & Linder, 2014; Linder, 2014). The inferred colonization of the Afrotropics from the Palaearctic seems to have taken place during the late Miocene (Table 2, Fig. 5). It coincides with three major events that affected the area: (1) worldwide cooling (Schuster *et al.*, 2006); (2) increasing aridity in northern Africa, which led to the origin of the Sahara Desert (11.6–7.2 Ma; Zhang *et al.*, 2014); and (3) the uplift of the Eastern Arc Mountains (8–7 Ma; Sepulchre *et al.*, 2006). It should be noted that our results do not rule out colonization of east Africa from the Palaearctic with the Arabian mountains as stepping-stones and subsequent extinction in this part of SW Asia. This alternative vicariance hypothesis has been demonstrated in other plant groups (Assefa *et al.*, 2007; Popp *et al.*, 2008; Thiv *et al.*, 2010; Mairal *et al.*, 2015; Pokorny *et al.*, 2015). However, under this scenario, we would have expected a closer relationship of the African plants to lineage B1 of *C. pendula*, as this lineage was presumably present in SW Asia during the period the colonization occurred. In addition, none of our inferred biogeographical scenarios invokes the past presence of the African lineage in SW Asia.

Interestingly, colonization of the Afrotemperate regions via Madagascar as suggested as one possible scenario in our analyses has not been reported in the literature before (Fig. 2). In addition, the haplotype network (Fig. 4B) suggests the presence of the ancestral haplotype (H11; Fig. 4) in two disjunct areas of mainland Africa (Fig. 1): the Simien Mountains (Ethiopia) and the Drakensberg (eastern South Africa). Moreover, two haplotypes which occur in Madagascar appear to be derived from those on the mainland (Fig. 4). This points to an initial colonization of eastern Africa and subsequently dispersal to Madagascar (at least twice), instead of the other way round as represented in the most likely ancestral area reconstruction (Fig. 2).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. List of studied material.
Appendix S2. Supplementary figures.
Appendix S3. Additional methodological details.

BIOSKETCH

Mónica Míguez is a Ph.D student at Pablo de Olavide University, Seville (Spain). Her research is focused on the evolution of angiosperms, with special interest in the systematics and biogeography of the genus *Carex* (Cyperaceae).

Author's contributions: P.J.-M. and S.M.-B. conceived the idea, collected plant material and drafted the manuscript; E.M. and B.G. analysed data and collected plant material; M.M. carried out the laboratory work, analysed the data and led the writing. All authors contributed to the writing of the final version.

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SUPPORTING INFORMATION

Carex sect. *Rhynchocystis* (Cyperaceae): a Tertiary subtropical relict in the western Palearctic showing a dispersal derived Rand-Flora Pattern

Mónica Míguez, Berit Gehrke, Enrique Maguilla, Pedro Jiménez-Mejías, Santiago Martín-Bravo

Appendix S1 List of studied material. Table S1

Table S1 List of studied material including species and "botanical country" as in Brummitt (2001) where each sample was collected, locality of collection, voucher, ITS and ETS NCBI GenBank accession numbers, matK and rpl32-trnLUAG NCBI GenBank accession numbers , ribotype and haplotype number.

Species (botanical country)	Country: locality	Voucher/herbarium code	ITS /ETS Accession number	*mat*K/ *rpl*32-*trn*L^{UAG} Accession number	Ribotype/ Haplotype
Section *Rhynchocystis* Dumort.					
Carex bequaertii De Wild.					
C. bequaertii (ETH 1)	Ethiopia, Bale Mountains National Park. Gaysay Valley.	B.Gehrke et al., *BG241* (Z-000081204)	ITS (EU288575) ETS (KU939528)	*mat*K(KU939683) *rpl*32-*trn*L (KU939763)	R6/H11

C. bequaertii (ETH 2)	Ethiopia, Oromia, Asella.	W.J.J.O de Wilde & B.E.E.de Wilde-Duyfjes, *6598* (P-01825505)	ITS (KU939605) ETS (KU939529)	*mat*K (KU939684)	R4
C. bequaertii (ETH 3)	Ethiopia, Bale Mountains National Park, Gaysay Valley.	B. Gehrke, *BG240 (Z)*	ITS (KU939606) ETS (KU939530)	*mat*K (KU939685) *rpl*32-*trn*L (KU939764)	R5/H11
C. bequaertii (KEN 1)	Kenya, Aberdares National Park. Oldonyio Lesatima.	M.Muasya & B.Gehrke, *BG14* (Z-000081207)	ITS (EU288571) ETS (KU939531)	*mat*K (KU939686)	R8
C. bequaertii (KEN 2)	Kenya, Mount Kenya National Park. Naro Moru route.	M.Muasya & B.Gehrke, *BG79* (Z-000081200/01)	ITS (EU288572) ETS (KU939532)	*mat*K (KU939687) *rpl*32-*trn*L (KU939765)	R8/H12
C. bequaertii (KEN 3)	Kenya, Mount Kenya National Park. Sirimon Route path.	M. Muasya & B.Gehrke, *BG98* (Z-000081202)	ITS (EU288573) ETS (KU939533)	*mat*K (KU939688) *rpl*32-*trn*L (KU939766)	R10/H13
C. bequaertii (KEN 4)	Kenya, Mount Elgon National Park. Koroborte.	M.Muasya & B.Gehrke, *BG145* (Z-000081203)	ITS (EU288574) ETS (KU939534)	*mat*K (KU939689) *rpl*32-*trn*L (KU939767)	R7/H14
C. bequaertii (KEN 5)	Kenya, Aberdares National Park, Mutubio.	M.L.Buide et al., *82UPO-K* (UPOS-3288-1)	ITS (KU939611) ETS (KU939535)	*mat*K (KU939690)	R9
C. bequaertii (KEN 6)	Kenya, Cherangani Hills, Kaibwibich.	Thulin & Tidigs, *49* (UPS-V571756)		*rpl*32-*trn*L (KU939768)	
C. bequaertii (UGA)	Uganda, Rwenzori Mountains, between Nyabitaba Hut and John Matte Hut.	B.Gehrke & H.P.Linder *BG352* (Z-000081205)	ITS (KU939612) ETS (KU939536)	*mat*K (KU939691)	R4
Carex microcarpa Bertol. ex Moris.					
C. microcarpa (COR 1)	Francia, Corsica, Asco.	M.Escudero & M.Luceño, *104ME07* (UPOS-4723)	ITS (KU939613) ETS (KU939537)	*mat*K (KU939692) *rpl*32-*trn*L (KU939769)	R35/H17
C. microcarpa (COR 2)	Francia, Corsica, Ghisome.	M.Escudero & M. Luceño, *88ME07*(UPOS-4730)	ITS (KU939614) ETS(KU939538)	*mat*K (KU939693) *rpl*32-*trn*L (KU939770)	R35/H17
C. microcarpa (COR 3)	Francia, Corsica, Muracciole.	M.Escudero & M. Luceño, *83ME07*(UPOS-4726)	ITS(KU939615) ETS(KU939539)	*mat*K (KU939694) *rpl*32-*trn*L (KU939771)	R36/H17
C. microcarpa (SAR 1)	Italy, Sardinia, Ogliastra.Rio Pira.	Urbani & Calvia, (SS)		*mat*K (KU939695) *rpl*32-*trn*L (KU939772)	H17
C. microcarpa (SAR 2)	Italy, Sardinia, Ogliastra. Baumela river.	Urbani & Calvia, (SS)	ITS (KU939616) ETS (KU939540)	*mat*K (KU939696) *rpl*32-*trn*L (KU939773)	R35/H17
Carex mossii Nelmes.					

C. mossii (CPP 1)	South Africa, Eastern Cape, Hogsback, Auckland Forest Reserve.	C.Reid, *Reid1204* (UPOS-3080)	ITS (KU939621) ETS (KU939547)	*mat*K (KU939702) *rpl*32-*trn*L (KU939777)	R2/H11
C. mossii (CPP 2)	South Africa, Eastern Cape, Hogsback, Auckland Forest Reserve.	C.Reid, *Reid1204* (Z-000081216)	ITS (KU939622) ETS (KU939548)	*mat*K (KU939703)	R3
C. mossii (NAT 1)	South Africa, Kwazulu-Natal, Bushman´s Nek, Caravan Park.	S.Martín-Bravo et al., *169SMB08* (UPOS)	ITS (KU939617) ETS (KU939541)	*mat*K (KU939697) *rpl*32-*trn*L (KU939774)	R1/H11
C. mossii (NAT 2)	South Africa, Kwazulu-Natal, Monk´s cowl Natural Park.	M.Luceño et al., *73ML08* (UPOS-4725)	ITS (KU939618) ETS (KU939542)	*mat*K (KU939698) *rpl*32-*trn*L (KU939775)	R1/H11
C. mossii (NAT 3)	South Africa, Kwazulu-Natal, Ntabamhlophe.	E.Maguilla et al., *50EMS12* (UPOS-5041)	ITS (KU939619) ETS (KU939543)	*mat*K (KU939699) *rpl*32-*trn*L (KU939776)	R1/H11
C. mossii (NAT 4)	South Africa, Kwazulu-Natal, Pongola Bush Nature reserve.	C.Reid, *Reid2432* (PRE-762613)	ETS (KU939544)	*mat*K (KU939700)	
C. mossii (NAT 5)	South Africa, Kwazulu-Natal, Giants Castle Nature Reserve.	C.Reid, *Reid983* (PRE-762269)	ITS (KU939620) ETS (KU939545)		R1
C. mossii (NAT 6)	South Africa, Kwazulu-Natal, Weza Forest Reserve.	T.K.Lowrey, *60417* (PRE-762302)	ETS(KU939546)	*mat*K (KU939701)	
C. mossii (TVL 1)	South Africa, Transvaal, Barterton.	F.A..Rogers, *30212* (Z-000080356)	ITS (KU939623) ETS(KU939549)	*rpl*32-*trn*L (KU939778)	R1/H11
C. mossii (TVL 2)	South Africa, Transvaal, Ohrigstad Dam Nature Reserve.	N.Jacobson, *Jacobson1790* (PRE-019484)	ITS (KU939624)	*mat*K (KU939704)	
Carex pendula Huds.					
C. pendula (AUT 1)	Austria, village Unterach am Attersee, SW of Stockwinkl.	E.Vitek, *96-244* (M-0177712)	ITS (KU939625) ETS (KU939550)	*rpl*32-*trn*L (KU939779)	R20/H9
C. pendula (AUT 2)	Austria, Vorarlberg.	W.Lippert, *15024* (M-0177708)	ITS (KU939626) ETS (KU939551)	*mat*K (KU939705) *rpl*32-*trn*L (KU939780)	R21/H9
C. pendula (AUT 3)	Austria, Tirol, Wallchsee-Kranzach.	W.Lippert, *166563* (M-0177709)	ITS (KU939627) ETS (KU939552)	*mat*K (KU939706) rpl32-trnL (KU939781)	R20/H9
C. pendula (AZO 1)	Portugal, Azores, Ilha do Pico.	H.Schaefer, *Shaefer2013/89* (TUM)	ITS (KU939628) ETS (KU939553)	*mat*K (KU939707) *rpl*32-*trn*L (KU939782)	R24/H2
C. pendula (AZO 2)	Portugal, Azores, Santa Maria.	H.Schaefer, *Shaefer2013/90* (TUM)	ITS (KU939629) ETS (KU939554)	*mat*K (KU939708) *rpl*32-*trn*L (KU939783)	R24/H2
C. pendula (BEL)	Belgium, Liège Seraing.	J. Lambinon, *99/248* (M-0177701)	ITS (KU939630)	*mat*K (KU939709)	R17/H1

			ETS (KU939555)	*rpl*32-*trn*L (KU939784)	
C. pendula (BUL)	Bulgaria, Burgas M.StrandZa.	N.Vihodcevsky, *s.n.* (V-571923)		*rpl*32-*trn*L (KU939785)	
C. pendula (CYP)	Cyprus, environs de Stavros-tis-Psokas.	G.Alziar, *0977* (SEV-251911)	ITS (KU939631) ETS (KU939556)	*mat*K (KU939710) *rpl*32-*trn*L (KU939786)	R22/H5
C. pendula (CZE)	Czech Republic, Moravia Centralis.	J.Dvorák, *s.n.* (M-0151978)	ITS (KU939632) ETS (KU939557)	*mat*K (KU939711) *rpl*32-*trn*L (KU939787)	R20/H9
C. pendula (DEN)	Denmark, Sydjylland.	I.Segelberg, *s.n.* (V-181569)	ITS (KU939633) ETS (KU939558)	*rpl*32-*trn*L (KU939787)	
C. pendula (FRA 1)	France, Haute-Normandie, Eure.	P.Jiménez-Mejías *15PJM10* (UPOS-4099)	ITS (KU939634) ETS (KU939559)	*mat*K (KU939712) *rpl*32-*trn*L (KU939789)	R11/H3
C. pendula (FRA 2)	France, Cevennes.	P.Jiménez-Mejías *104PJM09* (UPOS-5878)	ITS (KU939635) ETS (KU939560)	*mat*K (KU939713) *rpl*32-*trn*L (KU939790)	R16/H1
C. pendula (FRA 3)	France, Eurodisney. Marne-la-Vallée, (cultivated).	M.Míguez, *2MMR12* (UPOS-5197)	ITS (KU939636) ETS (KU939561)	*mat*K (KU939714) *rpl*32-*trn*L (KU939791)	R15/H1
C. pendula (FRA 4)	France, Dax.	P. Jiménez-Mejías & M.Escudero, *17PJM06* (UPOS-4739)	ITS (KU939637) ETS (KU939562)	*mat*K (KU939715) *rpl*32-*trn*L (KU939792)	R11/H1
C. pendula (GEO 1)	Georgia, Keda Region, some 30 km east of Batumi.	M.Merello et al, *2299* (E-00281678)	ITS (KU939638)	*mat*K (KU939716) *rpl*32-*trn*L (KU939793)	
C. pendula (GEO 2)	Georgia, Abhaeskaya ASSR. Adzharis-Tskhali-env.	E.E.Gogina,*1521* (MHA)	ITS (KU939639)	*mat*K (KU939717) *rpl*32-*trn*L (KU939794)	H8
C. pendula (GEO 3)	Georgia, Kvareli Dagodehsky zapoednik. Gorge Lagodehis-tskapi.	E.E.Gogina, *2112* (MHA)	ITS (KU939640)	*mat*K (KU939718) *rpl*32-*trn*L (KU939795)	H8
C. pendula (GER 1)	Germany, Bayern. Thalkirchen, Golfplatz.	H.Förther, *4868* (M-0177739)	ITS (KU939641) ETS (KU939563)	*mat*K (KU939719) *rpl*32-*trn*L (KU939796)	R11/H4
C. pendula (GER 2)	Germany, Kreis traunstein.	W.Lippert, *MTB8142/3* (M-0177733)	ITS (KU939642) ETS (KU939564)	*mat*K (KU939720) *rpl*32-*trn*L (KU939797)	R20/H9
C. pendula (GER 3)	Germany, Black forest Kandern.	Fr.Hörl, *s.n.* (M-0177726)	ITS (KU939643) ETS (KU939565)		R20
C .pendula (GER 4)	Germany, Baden-Württemberg, Heidelberg.	K.P.Buttler, *11244* (M-0177720)	ITS (KU939644) ETS (KU939566)	*mat*K (KU939721) *rpl*32-*trn*L (KU939798)	R33/H1

C. pendula (GER 5)	Germany, Bavaria, Kreis Freising; St. Alban	J.Sellma, MTB 7837/3 (M-0177729)	ITS (KU939645) ETS (KU939567)	*mat*K (KU939722) *rpl*32-*trn*L (KU939799)	R20/H9
C. pendula (GER 6)	Germany, Bavaria, Kreis Unterllgäu.	W.Lippert, *20008* (M-0177736)	ETS (KU939568)	*mat*K (KU939723) *rpl*32-*trn*L (KU939800)	H10
C. pendula (GER 7)	Germany, Bavaria, Amberg-Sulzbach district.	W Lippert, *16864* (P-01707382)	ETS (KU939569)	*mat*K (KU939724) *rpl*32-*trn*L (KU939801)	H4
C. pendula (GRB 1)	United Kingdom, Devon, Slapton Ley Field Centre.	S. Watson-Jones, *s.n.* (MTMG)	ITS (AY757600) ETS(AY757661)	*mat*K (KU939725) *rpl*32-*trn*L (KU939802)	R11/H1
C. pendula (GRB 2)	United Kingdom, Hertfordshire, The Moors, Braughing.	T. James, *TL385255* (Personal herbarium)	ITS (KU939647) ETS (KU939571)	*mat*K (KU939726) *rpl*32-*trn*L (KU939803)	R11/H1
C. pendula (GRB 3)	United Kingdom, London, Hampsted, Heath, Lake Ken Wood.	M.A.Spencer, *MAS/2012/040* (UPOS-5004)	ITS (KU939648) ETS (KU939572)	*mat*K (KU939727) *rpl*32-*trn*L (KU939804)	R11/H1
C. pendula (GRC)	Greece, Epiro, Koroni.	P.Vargas & M. Luceño, *261PV04* (UPOS-815)	ITS (KU939649) ETS (KU939573)	*mat*K (KU939728) *rpl*32-*trn*L (KU939805)	R14/H5
C. pendula (IRA)	Iran, Gilan.	Kukkonen, *39760* (M-015970)	ITS (KU939651) ETS (KU939575)	*rpl*32-*trn*L (KU939807)	R19/
C. pendula (ITA 1)	Italy, Elba, Marciana Marina.	Laubwald, *s.n.* (M-0177704)	ITS (KU939652)	*mat*K (KU939730) *rpl*32-*trn*L (KU939808)	H1
C. pendula (ITA 2)	Italy, Tuscany, Alpi Apuane, Vinca.	P.Jiménez-Mejías, *24PJM10* (UPOS-4136)	ITS (KU939654)	*mat*K (KU939732) *rpl*32-*trn*L (KU939810)	H1
C. pendula (ITA 3)	Italy, Torino, Puente de Valle Ceppi.	P.Jiménez-Mejías et al., *105bisPJM12* (UPOS)	ITS (KU939653) ETS (KU939576)	*mat*K (KU939731) *rpl*32-*trn*L (KU939809)	R11/H1
C. pendula (KRI)	Greece, Crete, Jania, Langos.	S.Martín-Bravo & M. Luceño, *378SMB05* (UPOS-242)	ITS (KU939655) ETS (KU939577)	*mat*K (KU939733 *rpl*32-*trn*L (KU939811)	R27/H5
C. pendula (MOR)	Morocco, Chefchaouèn.	M.Ait Lafkih et al., *61* (BM-340)	ITS (KU939658) ETS (KU939582)	*mat*K (KU939736) *rpl*32-*trn*L (KU939814)	R28/H6
C. pendula (MDR 1)	Portugal, Madeira, Levad Tower in Vamara.	Nobrega, *273* (MADJ-5494)	ETS (KU939578)		
C. pendula (MDR 2)	Portugal, Madeira, Santana.	M.Sequeira, *MS7806* B (UPOS-5182)	ITS (KU939656) ETS (KU939579)	*mat*K (KU939734) *rpl*32-*trn*L (KU939812)	R28/H7
C. pendula (POR 1)	Portugal, Cousá. Quinta de alfocheria.	R.Palhminha, *s.n.* (LISU-P7402)	ETS (KU939580)		
C. pendula (POR 2)	Portugal, Sintra.	J. C. Zamora, s.n. (MA)	ITS (KU939657)	*mat*K (KU939735)	R29/H1

			ETS (KU939581)	*rpl*32-*trn*L (KU939813)	
C. pendula (SIC)	Italy, Sicily, Syracuse, Sortino Valle dell`Ánapo.	S.F.M. Raimondo et al, *2156* (SEV-263402)	ITS (KU939659) ETS (KU939583)	*mat*K (KU939737) *rpl*32-*trn*L (KU939815)	R13/H1
C. pendula (SER)	Serbia, Bor District, Djerdap National Park. Miroc Mountains.	P.Jiménez-Mejías, *86PJM10* (UPOS-4208)	ITS (KU939660) ETS (KU939584)	*mat*K (KU939738) *rpl*32-*trn*L (KU939816)	R20/H9
C. pendula (SPA 1)	Spain, Asturias, Grado, La Mata.	S.Martín-Bravo et al., *637SMB05* (UPOS-2793)	ITS (KU939661) ETS (KU939585)	*mat*K (KU939739) *rpl*32-*trn*L (KU939817)	R32/H6
C. pendula (SPA 2)	Spain, Huelva, Road between San Telmo and N-485.	J.M.Marín, *904JMM* (UPOS-954)	ITS (KU939662) ETS (KU939586)	*mat*K (KU939740) *rpl*32-*trn*L (KU939818)	R31/H1
C. pendula (SPA 3)	Spain, Cádiz, Tarifa, Sierra del Bujeo.	P.Jiménez-Mejías, *90PJM04* (UPOS-2155)	ITS (KU939663) ETS (KU939587)	*rpl*32-*trn*L (KU939819)	R30/
C. pendula (SPA 4)	Spain, Jaén, Despeñaperros, Aldeaquemada road.	P.Jiménez-Mejías, *62PJM09* (UPOS-4720)	ITS (KU939664) ETS (KU939588)	*mat*K (KU939741) *rpl*32-*trn*L (KU939820)	R18/H1
C. pendula (SPA 5)	Spain, Cáceres, Navalconcejo.	P Jiménez-Mejías et al., *52PJM11* (UPOS-4719)	ETS (KU939589)	*mat*K (KU939742) *rpl*32-*trn*L (KU939821)	H1
C. pendula (SPA 6)	Spain, Sevilla, Cazalla de la Sierra, Isla Margarita.	P.Jiménez-Mejías, *61PJM03* (UPOS-5877)	ITS (KU939665) ETS (KU939590)	*mat*K (KU939743) *rpl*32-*trn*L (KU939822)	R26/H1
C. pendula (SPA 7)	Spain, Sevilla, Maria Luisa Park (cultivated).	E Maguilla & J.M.G.Cobos, *8EMS12* (UPOS-5194)	ITS (KU939666) ETS (KU939591)	*mat*K (KU939744) *rpl*32-*trn*L (KU939823)	R12/H1
C. pendula (SPA 8)	Spain, Asturias, Colunga. Playa de la Griega.	F.J.Fernández & R.L.Sánchez, *10FJF12 2/2* (UPOS-4973)	ITS (KU939667) ETS (KU939592)	*mat*K (KU939745) *rpl*32-*trn*L (KU939824)	R25/H6
C. pendula (SPA 9)	Spain, Cáceres, Gredos	E.Maguilla et al. *4EMS12bis* (UPOS-5143)	ITS (KU939668) ETS (KU939593)	*mat*K (KU939746)	H1
C. pendula (SWI1)	Switzerland, Geneva, Terre de Prégnu , pré de fauche.	R.Piñeiro-Portela, *s.n* (UPOS-2649)	ITS (KU939669) ETS (KU939594)	*mat*K (KU939747) *rpl*32-*trn*L (KU939825)	R11/H4
C. pendula (SWI2)	Switzerland, Lugano Mendrisio.	J.Höller, *s.n* (M-0177707)	ITS (KU939670) ETS (KU939595)	*mat*K (KU939748) *rpl*32-*trn*L (KU939826)	R11/H4
C. pendula (UKR)	Ukraine, Veliky Berezny.	A.K.Skvortsov (M-0151973)	ITS (KU939650) ETS (KU939574)	*mat*K (KU939729) *rpl*32-*trn*L (KU939806)	R20/H9
C. pendula (YUG_CR)	Croatia, Licko-senjska: Dalmatien, PlitviCke.	H.Lenander, *s.n* (UPS-V571926)	ITS (KU939671)	*mat*K (KU939749)	R34/H1

C. pendula (YUG_SL 1)	Slovenia, Podravksa, Ptju..	M.Thulinsn, *s.n* (UPS-V571925)	ETS (KU939596) ITS (KU939672) ETS (KU939597)	*rpl*32-*trn*L (KU939827) *mat*K (KU939750) *rpl*32-*trn*L (KU939828)	R20/H9
C. pendula (YUG_SL 2)	Slovenia, LjubljanaPekel-Schlucht, südlich Borovnica.	K.P.Buttler, *9566* (M-0177703)	ITS (KU939673) ETS (KU939598)	*mat*K (KU939751) *rpl*32-*trn*L (KU939829)	R20/H9
C. pendula (TCS 1)	Azerbaijan, Region Krasnoparsky, Tuapse.	P.A.Kapmconoba, *s.n* (MHA)	ITS (KU939674)	*mat*K (KU939752) *rpl*32-*trn*L (KU939830)	H8
C. pendula (TCS 2)	Azerbaijan, Lekoransky Zone. Alekseyevka.	V.G.Eropob, *s.n* (MHA)	ETS (KU939599)	*rpl*32-*trn*L (KU939831)	
C. pendula (TCS 3)	Azerbaijan.	N.V.Kostylëva & N.V.Kostyleva, *s.n.* (MHA)	ITS (KU939675)	*mat*K (KU939753) *rpl*32-*trn*L (KU939832)	H9
Carex penduliformis Cherm.					
C. penduliformis (MDG 1)	Madagascar, Antisirana.	C.Randrianarivelo & Torize, *391* (P-01874872)		*mat*K (KU939754) *rpl*32-*trn*L (KU939833)	H15
C. penduliformis (MDG 2)	Madagascar, Mahajanga Bealanana.	S.Wohlhauser et al., *795* (P-01874870)	ETS (KU939600)	*mat*K (KU939755) *rpl*32-*trn*L (KU939834)	H16
C. penduliformis (MDG 3)	Madagascar, Antsiranana, Sava, Andapa, Doany	C. Rakotovao et al., *2518* (P-01874871)	ETS (KU939601)		
Section *Spirostachyae* Drejer ex Baley					
Carex distans L.					
C. distans (IRA)	Iran, Azerbaijan, Paygham-Marzroud.	A..Rad, *s.n (*38662/1IRAN)	ITS(EU812723) ETS(KU939522)	*mat*K(KU939678(*rpl*32-*trn*L (KU939757)	
Carex punctata Gaud					
C. punctata (KRI)	Grecia, Crete, Chania.	S.Martín-Bravo & M.Luceño, *381SMB05* (UPOS-257)	ITS(DQ384178) ETS(KU939523)	*mat*K(KU939679) *rpl*32-*trn*L (KU939758)	
Section *Ceratocystis* Dumort					
Carex demissa Hornem**.**					
C. demissa (MOR)	Morocco, Rif.	P.Jiménez-Mejías et al., *93PJM07* (UPOS-3517)	ITS(JN634656) ETS(KU939524)	*mat*K(KU939680) *rpl*32-*trn*L (KU939759)	
Carex flava L.					
C. flava (NOR)	Norway, Laponia, Skjervoy.	M.Luceño & M.Guzmán, *4005ML* (UPOS-403)	ITS(AY278310) ETS(KU939525)	*mat*K(KU939681) *rpl*32-*trn*L (KU939760)	

Section *Phacocystis* Dumort				
Carex reuteriana Boiss				
C. reuteriana (SPA)	Spain, Cáceres.	P.Jiménez-Mejías, *57PJM07* (UPOS-5957)	ITS(KU939602) ETS(KU939520)	*mat*K(KU939676) *rpl*32-*trn*L (JN222833)
Carex trinervis Dumort.				
C. trinervis (SPA)	Spain, Huelva.	P.Jiménez-Mejías,*43PJM07*(UPOS-2205)	ITS(KU939603) ETS(KU939521)	*mat*K(KU939677) *rpl*32-*trn*L (KU939756)
Section *Sylvatica* Huds				
Carex sylvatica Huds.				
C. sylvatica (SWI)	Switzerland, forest near Basel.(ITS, ETS, *rpl*32-*trn*L) United Kingdom: Glamorgan (*mat*K)	Lechowicz, *s.n.* (MTMG) NMW175 (*mat*K)	ITS(AY757599) ETS(AY757660)	*mat*K(JN896090) *rpl*32-*trn*L (KU939761)
C. rainbowii Luceño, Jim. Mejías, M. Escudero & Martín-Bravo.				
C. rainbowii (NAT)	South Africa: Kwazulu-Natal, Cathedral Peak Area, Rainbow Gorge,	S.Martin-Bravo & M.Luceño, *120SMB11* (UPOS-5030)	ITS(KC122380) ETS(KC122388)	*mat*K(KU939682) *rpl*32-*trn*L (KU939762)

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SUPPORTING INFORMATION

Carex sect. *Rhynchocystis* (Cyperaceae): a Tertiary subtropical relict in the western Palearctic showing a dispersal derived Rand-Flora Pattern

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Appendix S2 Supplementary Figures including Majority-rule consensus trees of *Carex* sect. *Rhynchocystis* obtained from the Bayesian Inference analysis of the ETS region (Figure S1), ITS region (Figure S2), concatenated nrDNA matrix ETS-ITS (Figure S3) and concatenated ptDNA matrix *mat*K and *rpl*32-*trn*L^{UAG} (Figure S4). Species trees for *Carex* sect. *Rhynchocystis*, using post-burn-in trees from the Bayesian inference analyses of three markers (ETS, ITS, and *m*atK) and four marker (ETS, ITS, *mat*K and *rpl*32-*trn*L^{UAG}) in Figure S5 and Figure S6 respectively. Statistical parsimony network of 36 nrDNA ribotypes retrieved from the analysis of the combined ITS and ETS sequences in Figure S7.

Figure S1 Majority-rule consensus tree of *Carex* sect. *Rhynchocystis* (Cyperaceae) resulted from the Bayesian Inference analysis of the ETS region. Numbers below branches indicate maximum parsimony bootstrap (BS) support values, and numbers above branches indicates Bayesian posterior probability (PP) values. Tip labels indicate species name and code of the source region (in parenthesis), following "botanical countries" as in Brummitt (2001), and including a number when there are more than one sample from the same region. Scale bar indicates substitutions per site.

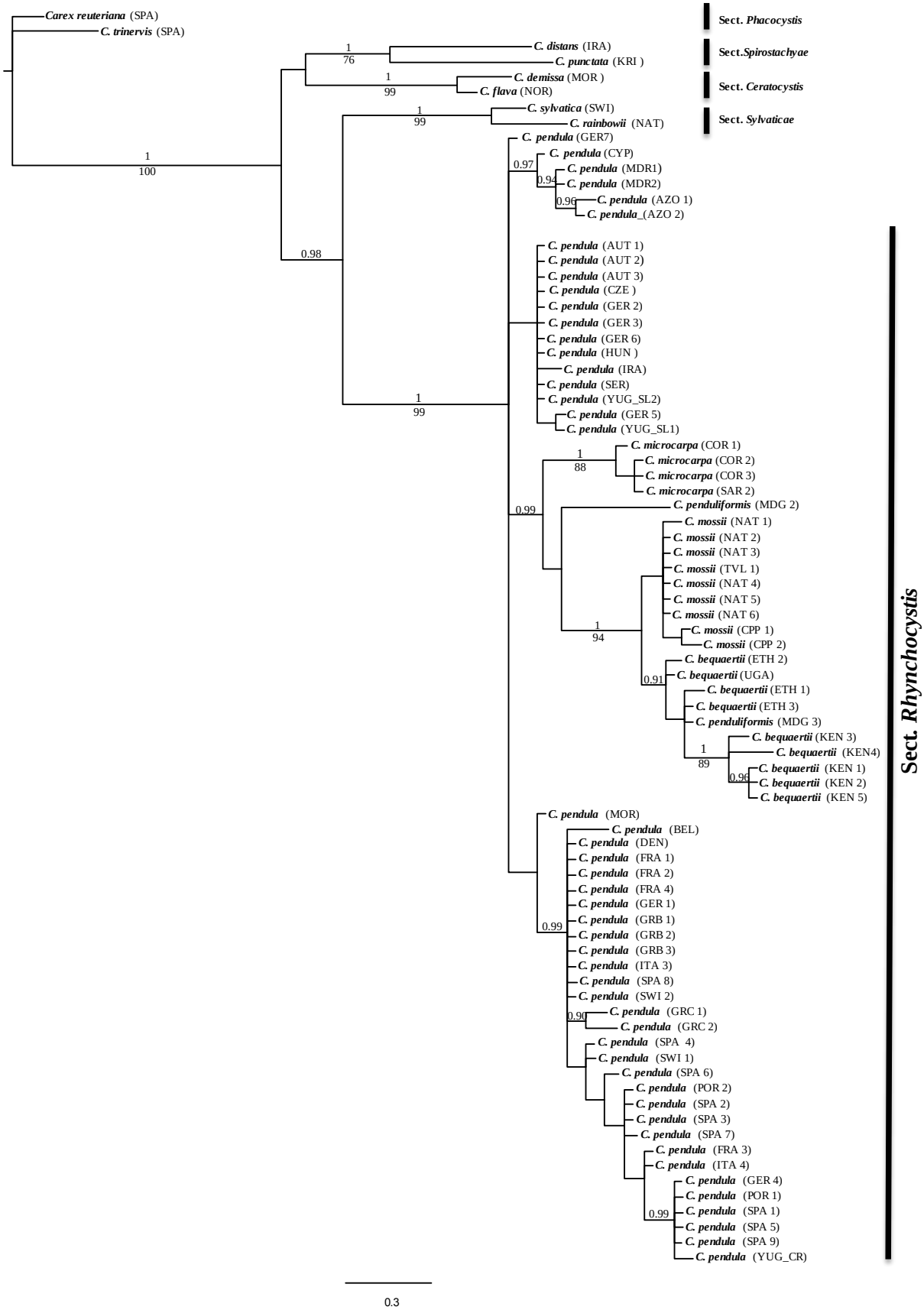


Figure S2 Majority-rule consensus tree of *Carex* sect. *Rhynchocystis* (Cyperaceae) resulted from the Bayesian Inference analysis of the ITS region. Numbers below branches indicate maximum parsimony bootstrap (BS) support values, and numbers above branches indicates Bayesian posterior probability (PP) values. Tip labels indicate species name and code of the source region (in parenthesis), following "botanical countries" as in Brummitt (2001), and including a number when there are more than one sample from the same region. Scale bar indicates substitutions per site.

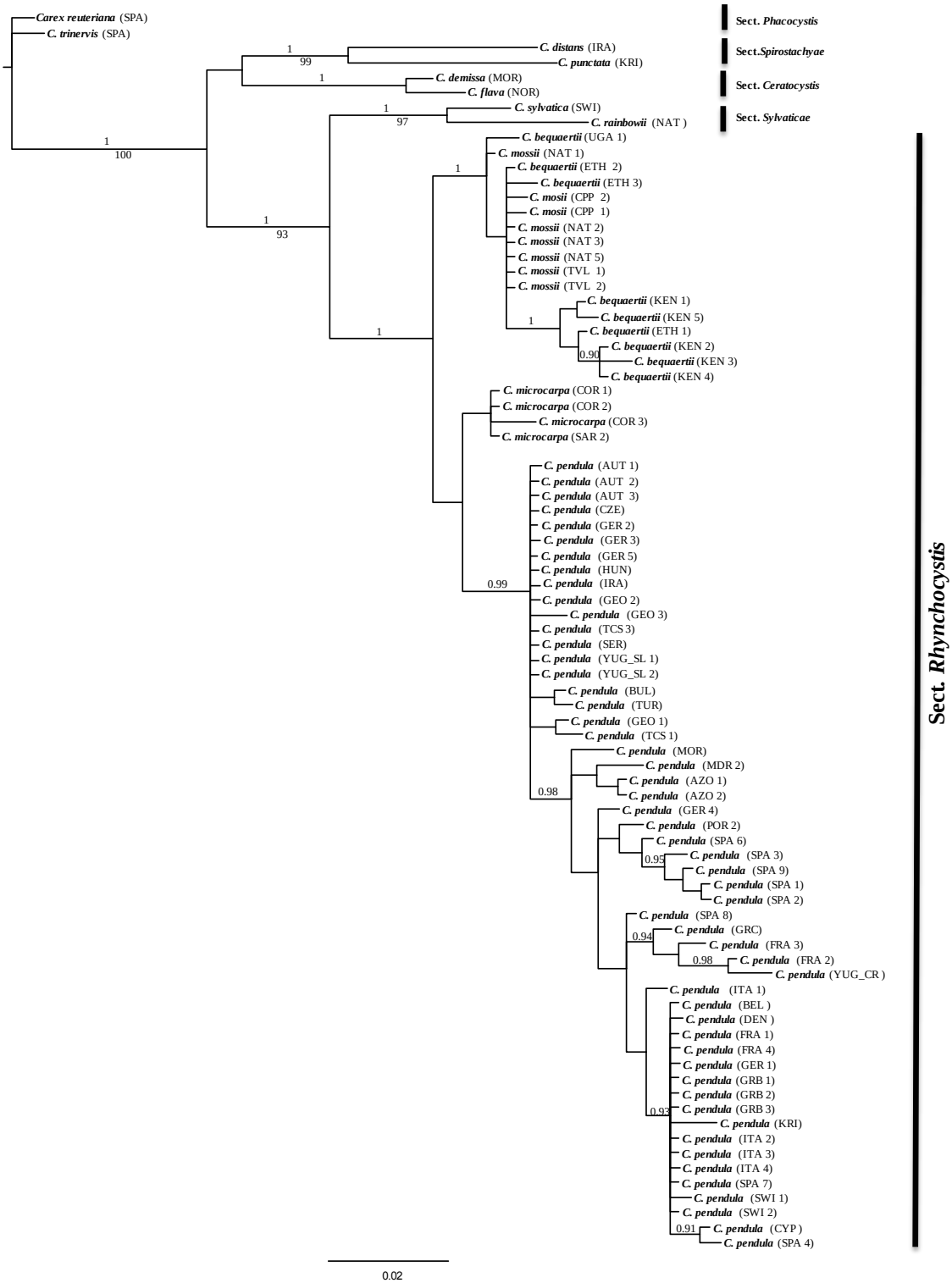


Figure S3 Majority-rule consensus tree of *Carex* sect. *Rhynchocystis* (Cyperaceae) resulted from the Bayesian Inference analysis of the concatenated matrix formed by the nrDNA regions ETS and ITS. Numbers below branches indicate maximum parsimony bootstrap (BS) support values, and numbers above branches indicates Bayesian posterior probability (PP) values. Tip labels indicate species name and code of the source region (in parenthesis), following "botanical countries" as in Brummitt (2001), and including a number when there are more than one sample from the same region. Scale bar indicates substitutions per site.

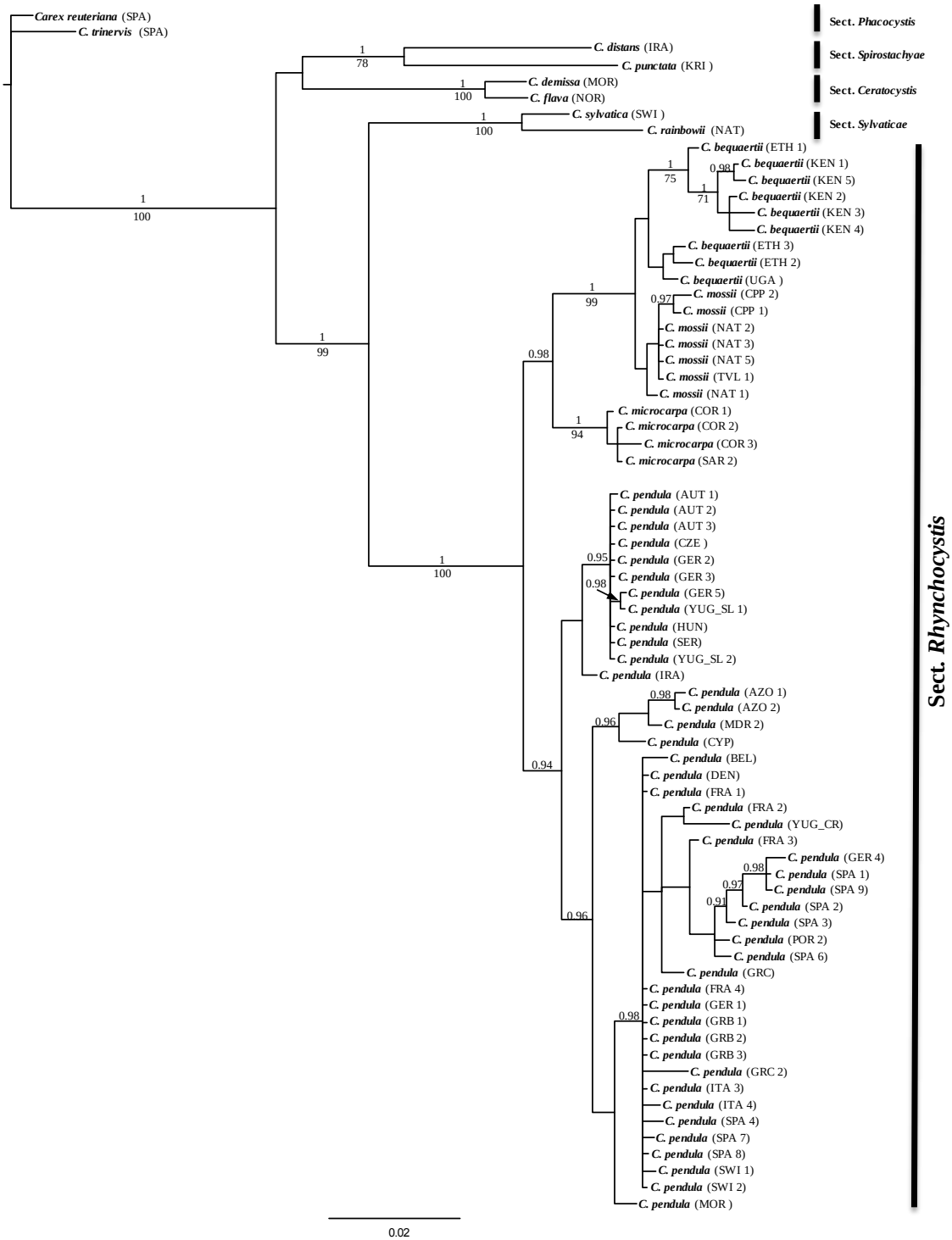


Figure S4. Majority-rule consensus tree of *Carex* sect. *Rhynchocystis* (Cyperaceae) resulted from the Bayesian Inference analysis of the concatenated matrix formed by the ptDNA regions *mat*K and *rpl*32-*trn*L^{UAG}. Numbers below branches indicate maximum parsimony bootstrap (BS) support values, and numbers above branches indicates Bayesian posterior probability (PP) values. Tip labels indicate species name and code of the source region (in parenthesis), following "botanical countries" as in Brummitt (2001), and including a number when there are more than one sample from the same region. Scale bar indicates substitutions per site.

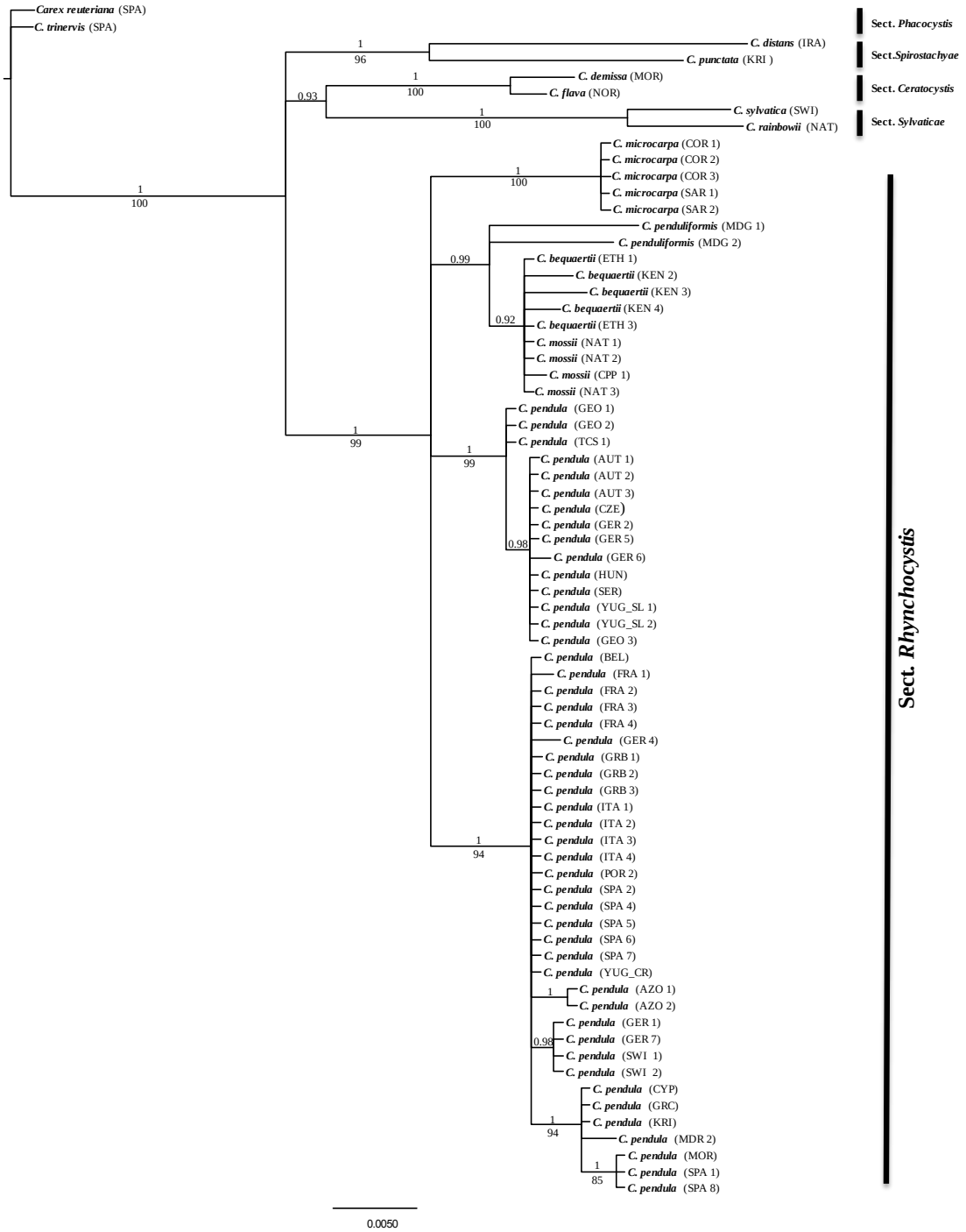


Figure S5. Species tree for section *Rhynchocystis*, using post-burn-in trees of three markers (ETS, ITS, and *m*atK) from the Bayesian inference analyses. Bootstrap supports (shown only if >50%) calculated from the bootstrapped individual gene-trees are shown above branches.
Tip labels indicate species name and code of the "botanical countries" where specimens were collected (in parenthesis), following Brummitt (2001) and including a number when there is more than one sample from the same region.

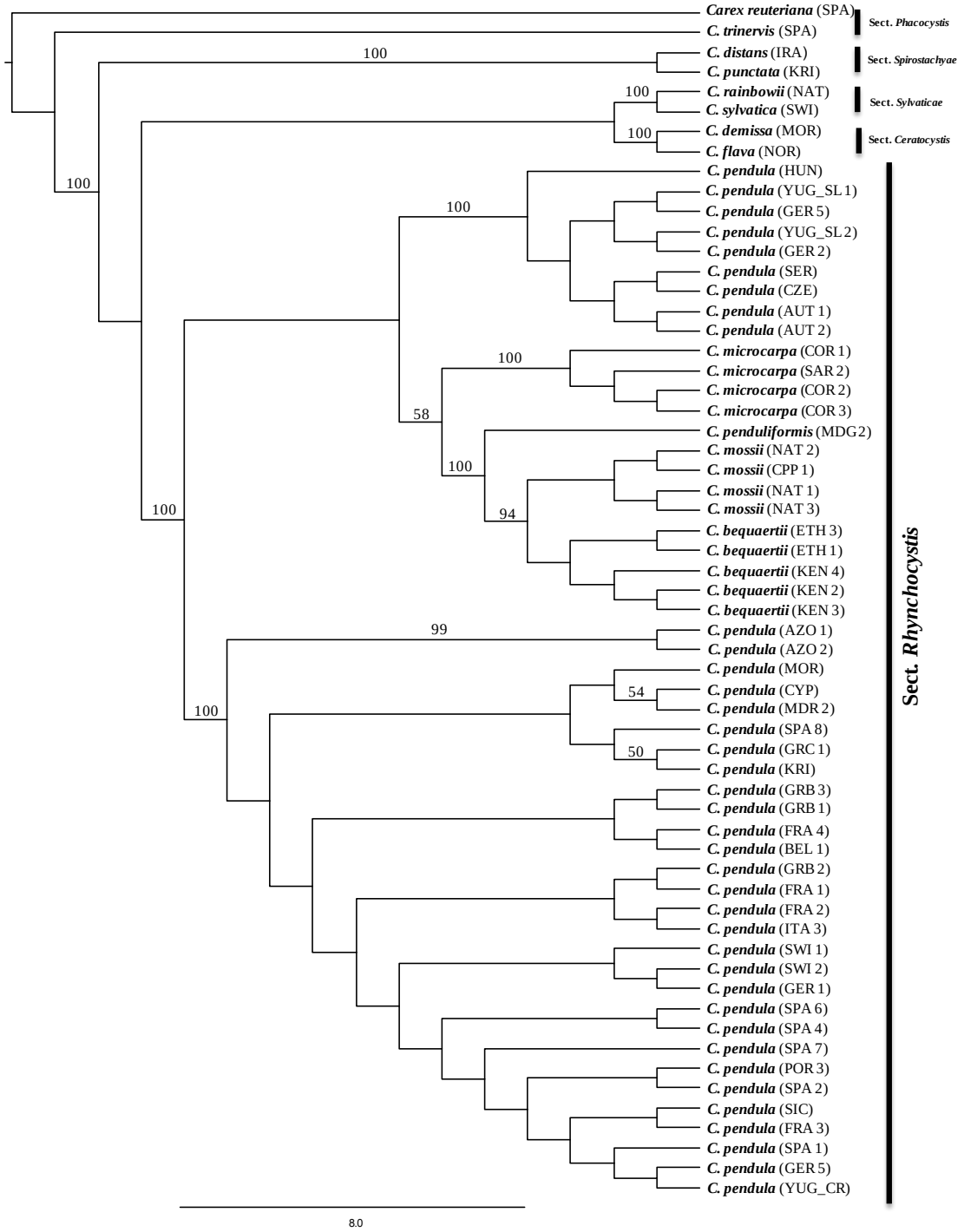


Figure S6 Species tree for section *Rhynchocystis* using post-burn-in trees of four markers (ETS, ITS, *mat*K and *rpl*32-*trn*L^{UAG}) from the Bayesian inference analyses. Carex penduliformis was excluded from this analysis due to the lack of ITS sequences. Bootstrap supports (shown only if >50%) calculated from the bootstrapped individual gene-trees are shown above branches. Tip labels indicate species name and code of the "botanical countries" where specimens were collected (in parenthesis), following Brummitt (2001) and including a number when there is more than one sample from the same region

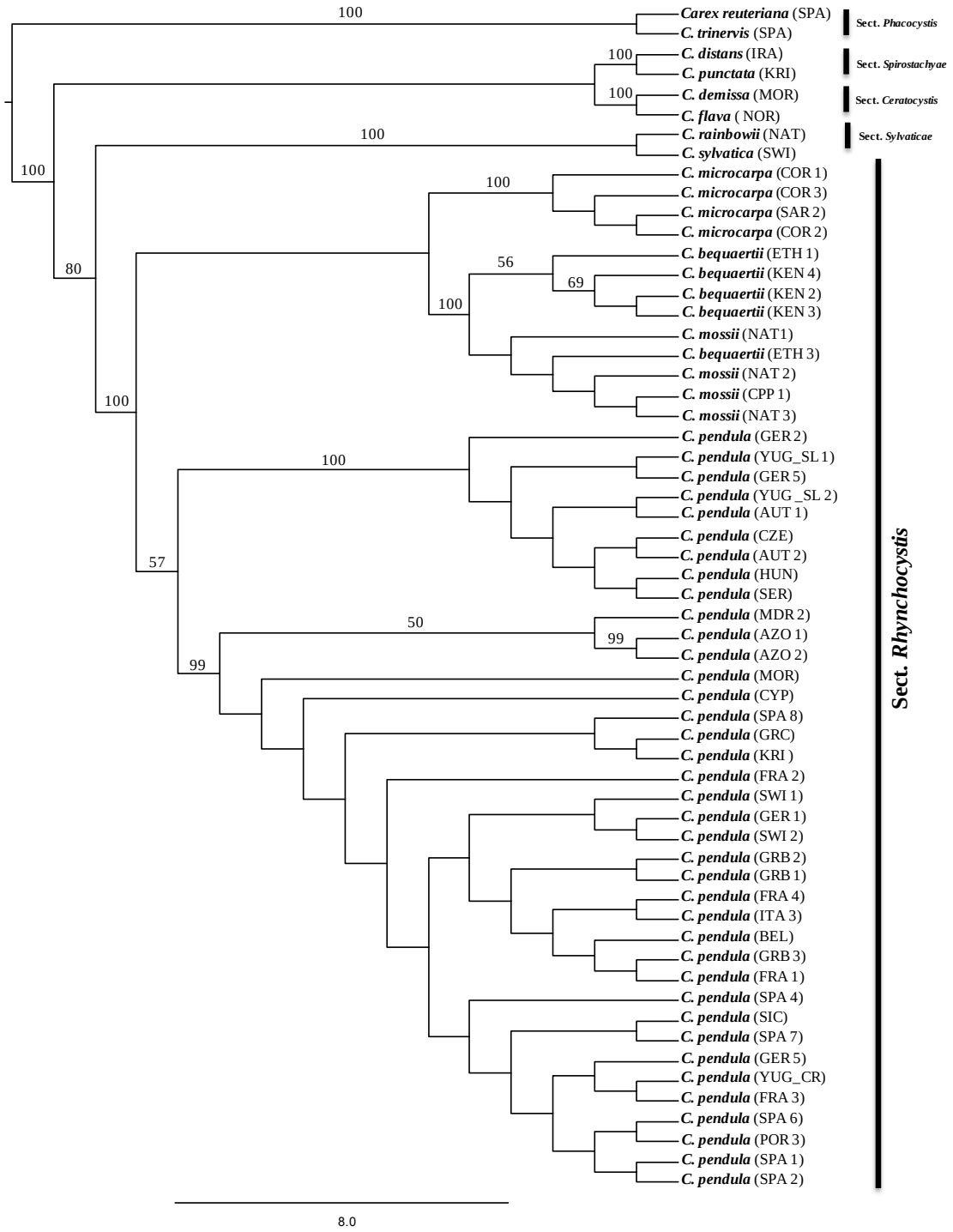
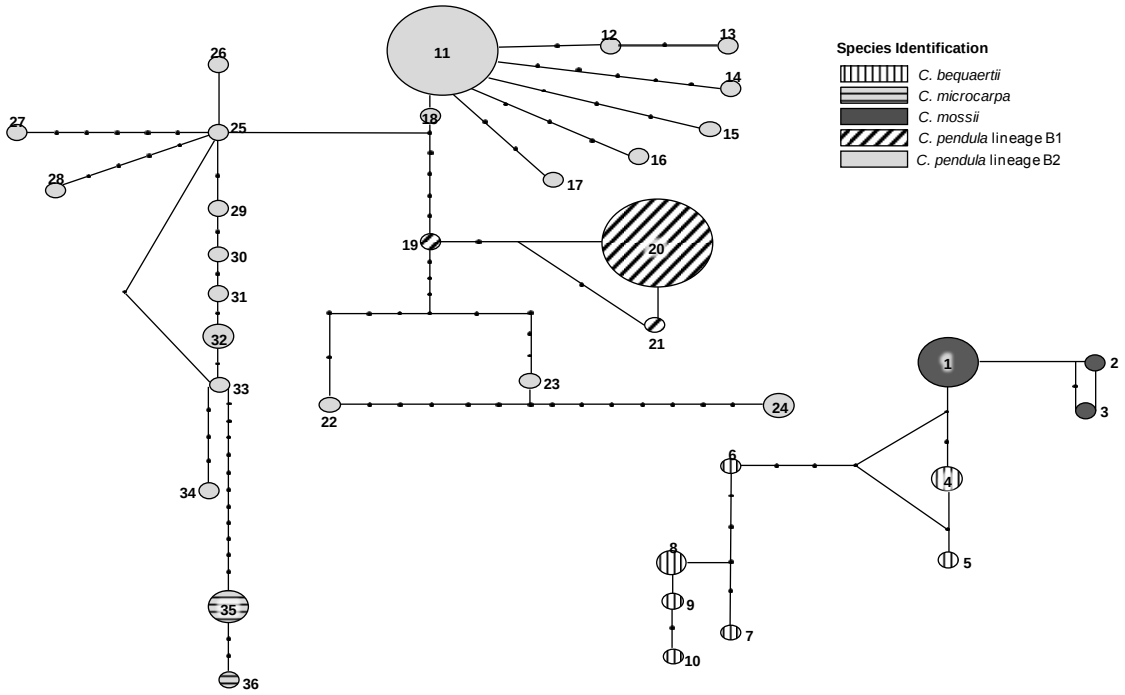


Figure S7 Statistical parsimony network of the 36 ribotypes retrieved from the analysis of the combined ITS and ETS sequences. Small black circles represent extinct or not sampled ribotypes, and each line between ribotypes represents a single mutation step. Circle size is proportional to the number of samples displaying the corresponding haplotype. Haplotypes from each population are given in Appendix S1.



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SUPPORTING INFORMATION

Carex sect. _Rhynchocystis_ (Cyperaceae): a Tertiary subtropical relict in the western Palearctic showing a dispersal derived Rand-Flora Pattern

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Appendix S3. Additional methodological details for the divergence time estimation analyses and BioGeoBEARS and BBMA details. Table S3.1: Primary calibration points. Figure S8: Maximum clade credibility phylogeny from the divergence time estimation analysis in *Carex* sect *Rhynchocystis* (see Fig. 5 for a summarized version of the tree). Area coded in the analyses (Figure S9). Results of BioGeoBEARS analyses (Table S3.2). Results of the BioGeoBEARS analysis (Figures S10, S11, S12, S13, S14 and S15).

Additional methodological details for the divergence time estimation analyses

We constructed a matrix of 64 combined ETS, ITS and matK sequences in total containing only a sequence per species (except for *C. pendula*, see below). Six sequences from sect. *Rhynchocystis* were included. We used two sequences for *C. pendula*, one from lineage B1 and another from lineage B2 because they form two different, strongly differentiated lineages in the phylogenetic-phylogeographic analyses (Figs. 3, 4). We also included 55 samples from the rest of *Car*ex, constituting a representative sampling of *Carex* main lineages, and three outgroups based on Carex Global Group (2015). The matrix had an aligned length of 1696 sites. A dated phylogeny was estimated using BEAST 1.8.0 (Drummond et al., 2012), using an uncorrelated log-normal relaxed clock model. The data set was not partitioned into individual genes (following Couvreur, 2010), and a general time reversible model without gamma rate distribution was applied. Two primary calibration points, based on fossil records, were applied as calibration points according to Jiménez-Mejías et al. (2016) (Table S5.1): 1- *Carex colwellensis* Chandler, aged 38-33.9 Ma (Priabonian, late Eocene). It is the oldest reliable known fossil ascribable to *Carex*. We used it to constrain the crown node of *Carex*. And 2- *C. limosioides* Negru, dated 23.03-15.97 Ma (early Miocene). It is the oldest reliable fossil of this species, which is considered to belong to section *Rhynchocystis*. Analyses were conducted using two independent Markov Chain Monte Carlo (MCMC) runs of 40 million generations each, we discarded the first 10% trees as burn-in. We included two

speciation process models: birth–death (Gernhard, 2008) and pure birth (Yule, 1924) as they have been shown to yield different age estimates (Gernhard, 2008). The Bayes factor as implemented in Tracer 1.5 (Rambaut and Drummond, 2009) was used to select the best-fitting model under the smoothed marginal likelihood estimate and with 1000 bootstrap replicates (Suchard et al., 2001). All BEAST analyses were performed on the computer cluster CIPRES Science Gateway 3.3 (http://www.phylo.org/). Tracer was used to assess graphically the convergence of runs and burn-in, and to check the ESS values for all parameters (indicated by ESS above 200). A maximum clade credibility (MCC) tree was calculated in TreeAnnotator 1.8.0 (Drummond et al., 2012), using a posterior probability limit of 0.7 and the mean heights option. Trees and parameters from the independent runs were combined with the program Logcombiner v. 1.8.0. Mean ages and 95% HPD intervals were retrieved with Tracer from the log combined file and visualized onto the MCC tree with the program FigTree v. 1.4.0.

Table S3.1. Primary calibration points based on fossils used in the dating analyses.

Lineage constrained (taxon set)	Fossil (Reference)	Age (Ma)	Zero offset	Log (SD)	Mean	5% Quantile	95% Quantile
Genus *Carex*	*C. colwellensis* (Chandler, 1963)	38-33.9	33.9	0.85	1.5	34.16	38.13
Carex section *Rhynchocystis*	*C. limosioides* (Negru, 1986)	23.03-15.97	15.97	1.8	2	15.99	23.61

References

Chandler, M. E. J. (1963) Revision of the Oligocene floras of the Isle of Wight. Bulletin of the British Museum (Natural History). *Geology* , 6, 321–384.

Couvreur T. L. P., Franzke A., Al-Shehbaz I.A., Bakker F.T., Koch M.A., Mummenhoff K. (2010). Molecular Phylogenetics, Temporal Diversification, and Principles of Evolution in the Mustard Family (Brassicaceae). *Molecular Biology and Evolution,* 27, 55–71.

Drummond A.J., Suchard M.A., Xie D., & Rambaut A. (2012) Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.

Gernhard T. (2008) The conditioned reconstructed process. Journal of Theoretical Biology,

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Rambaut A, Drummond AJ. 2009. Tracer version 1.5 ,. Available from http://beast.bio.ed.ac.uk .

Yule G. U. (1924). A mathematical theory of evolution based on the conclusions of Dr. J.C. Willis. Philosophical Transactions of the Royal Society London B, **213**, 21–87.

Negru, A. (1986) Meoticheskaya flora Severo-Zapadnogo Prichernomaria. Shiintsa, Chişinău.

Figure S.8 Maximum-credibility-clade phylogeny from the divergence time estimation analysis in *Carex* sect. *Rhynchocystis* under an uncorrelated lognormal clock model usinga combined matrix of ETS, ITS and *mat*K regions. Mean ages are shown above nodes. Node bars represent the 95% highest posterior density (HPD) intervals for the divergence time estimates of each node with posterior probabilities higher than 0.9. See Table 2 for ages and posterior probabilities inferred for clades.

Additional methodological BioGeoBEARS and BBMA details

Geographical range evolution was analysed using a model testing approach in the package BioGeoBEARS (Matzke, 2013, 2014) in R version 3.2.5. (R core team 2016). We compared the dispersal–extinction–cladogenesis (DEC) model utilized by Lagrange (Ree *et al*., 2005) and a likelihood implementation similar to DIVA (Ronquist, 1997).

As the geographical range evolution is likely to fit different models in the Afrotropics compared to the Palearctic (given contrasting disjunct versus contiguous distribution patterns respectively) we used two different strategies for area coding for the BioGeoBEARS analyses (see also Fig. S9 in Appendix S3**)**.

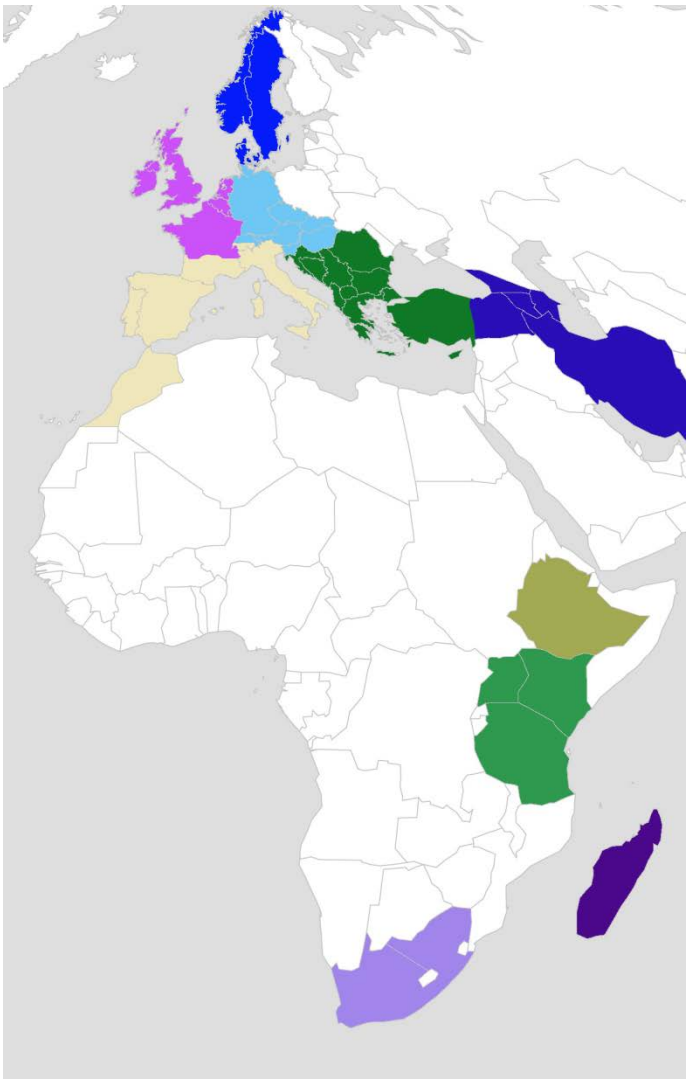
Areas coded for the scenario focusing on Africa:

a) Palearctic (i.e. non sub-Saharan areas);
b) Ethiopia;
c) Tropical East Africa;
d) Southern Africa;
e) Madagascar.

Areas coded for the scenario focusing on the Palearctic (pragmatically defined in part using political boundaries but reflecting climatic boundaries, e.g. within France, Switzerland and Turkey):

a) Northern Europe (Denmark, Norway, Sweden);
b) Western Europe (Belgium, Central and Northern France, British Isles, Netherlands);
c) Southern Europe and North-western Africa (Portugal, Spain, Southern France including Corsica, Italy including Elba and Sardinia, Southern Switzerland, as well as Morocco, Macaronesian Islands: Azores and Madeira);
d) South-eastern Europe (Greece, Cyprus, western Turkey as well as Croatia and Serbia);
e) Central Europe (Austria, Czech Republic, Germany, Hungary, Slovakia, Slovenia, Northern Switzerland);
f) Western Asia (Azerbaijan, Eastern Turkey, Georgia, Iran); and
g) Sub-Saharan Africa and Madagascar.

We also tested the influence of coding islands of the Tyrrhenian Sea (Corsica, Elba, Sardinia and Sicily) as separate entities in the Palearctic-focused scenario. We compared the results of the BioGeoBEARS analyses with and without a distance matrix based on the Paleartic



scenario and estimated whether founder event speciation was likely to have occurred (e.g. a daughter species occupies a range outside the distribution range of the ancestor). A comparison among all models (DEC, DEC+J, DIVA-LIKE, DIVA-LIKE+J) using the AIC was calculated to determine the best fitting model. The maximum number of areas allowed was set to 5 for the African analyses and 7 for all others.

For the Bayesian Binary MCMC Analysis (BBMA) as implemented in RASP 3.2 (Yu et al., 2015) we coded all regions for a single analysis:

a) Northern Europe: Denmark, Norway, Sweden;
b) Western Europe: Belgium, Central and Northern France, Great Britain, Netherlands;
c) Southern Europe and North-western Africa: Portugal, Spain, Southern France including, Italy, Southern Switzerland, as well as Morocco;
d) Macaronesian Islands of Azores and Madeira
e) Thyrrhenian Sea islands: Corsica, Elba and Sardinia;
f) South-eastern Europe: Greece including Crete and Cyprus, western Turkey as well as Croatia and Serbia;
g) Central Europe: Austria, Czech Republic, Germany, Hungary, Slovakia, Slovenia, Northern Switzerland;
h) Western Asia (Azerbaijan, Eastern Turkey, Georgia, Iran);
i) Ethiopia;
j) Kenya;
k) Uganda;
l) Southern Africa;
m) Madagascar

Figure S9.

Area coded in the analyses.
Light blue: Central Europe
Blue: Northern Europe
Dark Blue: Western Asia
Pink: Western Europe
Mauve: Southern Africa
Purple: Madagascar
Beige: South-eastern Europe
Ocra: Ethiopia
Green: East Africa
Dark green: South-eastern Europe

We used 1000 randomly chosen trees obtained from the posterior distribution from BEAST 1.7.5 (see above), in order to assess uncertainty in biogeographic reconstructions due to both topological and temporal uncertainty. We removed distant outgroup species before running the analyses and computed a consensus tree in RASP 3.2 to display the results. Bayesian Binary MCMC Analysis was set to: sample frequency of 1000, number of generations to 1,000,000, number of chains to 10, temperature to 0.1, and discharge of 500. The substitution model was set at a fixed JC with among site rate variation set to equal. Outgroup taxa represented by only a single accession were coded as multistate when applicable, and ingroup taxa as occurring at sampling locality only.

Tip labels indicate codes of the source regions, following "botanical countries" as in Brummitt (2001), and including a number when there is more than one sample from the same region as in Appendix S1.

Results

Biogeographical patterns using BioGeoBEARS

Alternative biogeographical models appear to result in similar biogeographic scenarios for *Carex* sect. *Rhynchocystis* (Appendix 3.Table S3.2; Figures S10-S13).

Table S 3.2 Results of BioGeoBEARS analyses. Paleartic=Eurasian centred scenario dividing Eurasia into 6 regions and coding sub-Saharan Africa and Madagascar as one area, without a distance matrix; Afrotropic= African centred scenario dividing Eurasia into four and sub-Saharan Africa also into four areas. +Dist= scenario using a distance matrix; Best model in each run indicated in bold. LnL = log likelihood, np= number of parameters**,** D = dispersal rate per million years along branches, e = extinction rate per million year along branches, j = founder event speciation weighted per speciation event.

	LnL	**np**	**D**	**e**	**j**	**AIC**	**AIC_wt**
Palearctic							
DEC	-161	2	0.029	1.00E-12	0	326	4.40E-18
DEC+J	**-137**	**3**	**0.019**	**1.00E-12**	**0.03**	**280**	**2.80E-08**
DIVA-LIKE	-137	3	0.023	1.00E-12	0.024	280	3.80E-08
DIVA-LIKE+J	-137	3	0.023	1.00E-12	0.024	280	3.80E-08
Palearctic + distances							
DEC	-161	2	0.029	1.00E-12	0	326	4.30E-14
DEC+J	-137	3	0.019	1.00E-12	0.03	280	0.0003
DIVA-LIKE	-137	3	0.023	1.00E-12	0.024	280	0.0004
DIVA-LIKE+J	**-137**	**3**	**0.023**	**1.00E-12**	**0.024**	**280**	**0.0004**
Palearctic with Tyrrhenian Sea							
DEC	-195	2	0.022	0.004	0	395	5.8e-12
DEC+J	-170	3	0.015	1e-12	0.024	347	0.15
DIVALIKE	**-169**	**3**	**0.017**	**4.4e-09**	**0.018**	**344**	**0.42**
DIVALIKE+J	**-169**	**3**	**0.017**	**1e-12**	**0.018**	**344**	**0.42**
Afrotropic							
DEC	-180	2	0.019	0.006	0	363	2.59E-22
DEC+J	**-153**	**3**	**0.010**	**1E-12**	**0.033**	**312**	**4.60E-11**
DIVA-LIKE	-155	3	0.013	3.70E-09	0.028	316	5.78E-12

DIVA-LIKE+J	-155	3	0.013	1E-12	0.028	316	5.78E-12
Afrotropic + distances							
DEC	-173	2	0.024	0.007	0	350	1.95E-19
DEC+J	**-150**	**3**	**0.012**	**1.0E-12**	**0.032**	**305**	**1.14E-09**
DIVA-LIKE	-152	3	0.016	4.9E-09	0.027	309	1.48E-10
DIVA-LIKE+J	-152	3	0.016	3.1E-09	0.027	309	1.48E-10

Most scenarios showed the highest likelihood for DEC models with founder-effect dispersal, with the exception of the Palearctic scenario coding islands in the Tyrrhenian Sea as separate areas. However, the likelihood scores of DIVA-LIKA models were very similar in all analyses to those of the DEC+J. The results indicate that sect. *Rhynchocystis* and the mrca of *C. pendula* and the African clade had a widespread distribution in the southern Palearctic. The two lineages of *C. pendula* survived at least since about 10 Ma and during the ice ages in separate refugia before recolonising Europe. The better fit of founder-effect dispersal in the DEC over DIVA-LIKE models indicates that Eastern Europe was more likely to have been colonised via dispersal instead of gradual range expansion. However, differences in model fit are very small and potentially reflects that both founder event speciation and gradual range expansion played a role at different times in the evolution of geographic distribution patterns in sect. *Rhynchocystis*.

The results of the BBMA analysis were very similar to the reconstruction of the BiogeoBEARS analyses (Fig. S15 in Appendix 3)

The sequence of colonisation of Africa was not reconstructed with certainty. An initial colonisation of Madagascar was reconstructed as the most likely place of origin in the Afrotropics with subsequent colonisation of continental Africa with Ethiopia or Southern Africa having nearly equal likelihood of having been colonised from Madagascar. However, the possibility of a colonisation of continental Africa and subsequent dispersal to Madagascar is not entirely ruled out by the results.

Using a distance model led to marginally better likelihood and AIC values but did not influence the biogeographic reconstructions significantly.

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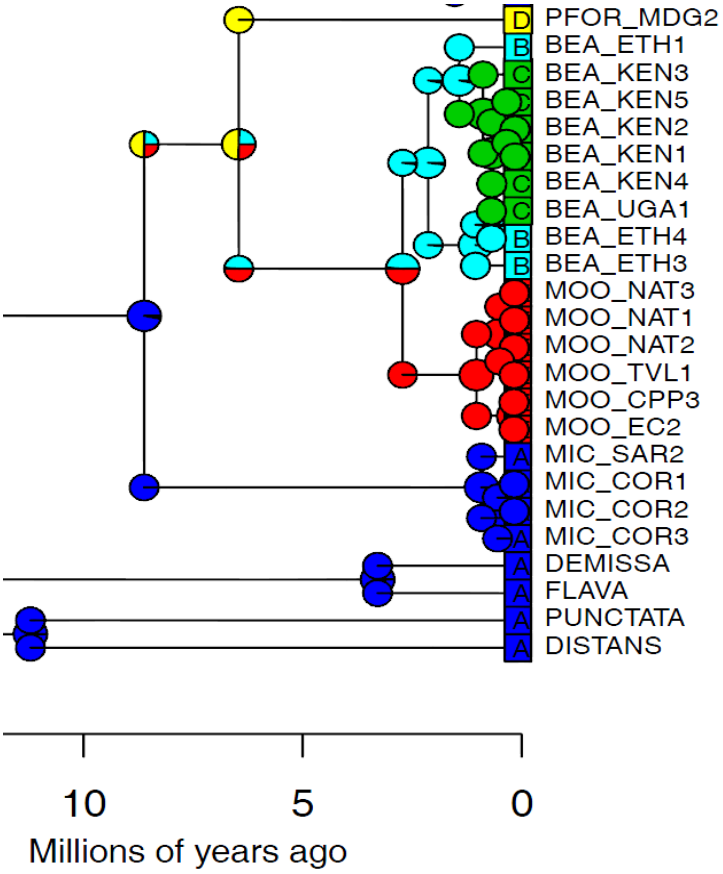
Figure S10.

Results of the BioGeoBEARS analysis for lineage A (*C. microcarpa*, *C. penduliformis*, *C. beaquaertii* and *C. mossii*; Fig. 3) using **DEC+J only coding Afrotropical regions** (A [dark blue] = Palearctic, B [light blue] = Ethiopia, C [green] =Tropical East Africa, D [yellow] = Madagascar, E [red] =Southern Africa).



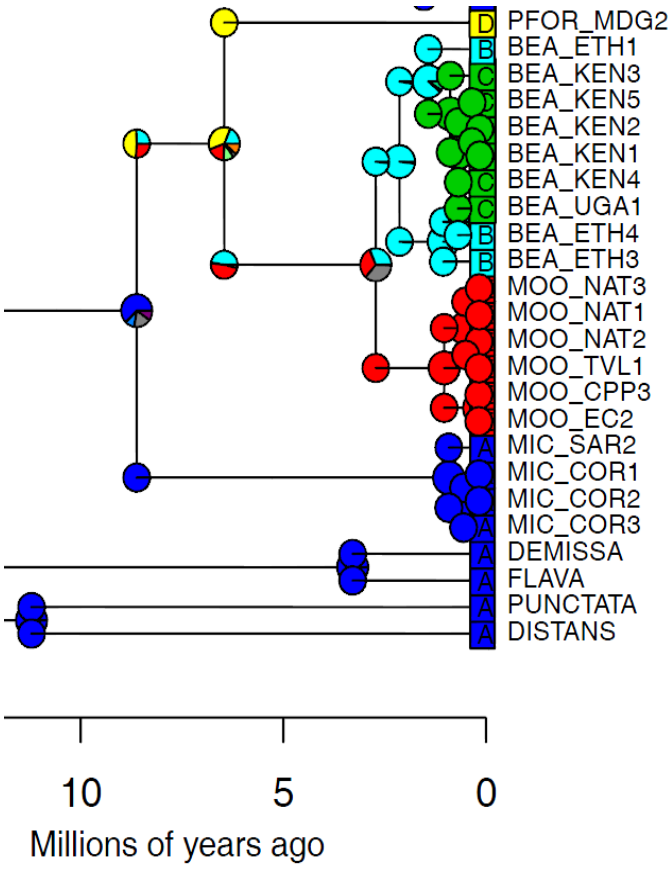


Figure S11.

Results of the BioGeoBEARS analysis for lineage A (*C. microcarpa*, *C. penduliformis*, *C. beaquaertii* and *C. mossii*; Fig. 3) using **DIVA-LIKE+J only coding Afrotropical regions** (A [dark blue] = Palearctic, B [light blue] = Ethiopia, C [green] =Tropical East Africa, D [yellow] = Madagascar, E [red] =Southern Africa; BE = grey, BDE = orange, BCDE = light green).

Figure S12.

Results of the BioGeoBEARS analysis for lineage A (*C. microcarpa*, *C. penduliformis*, *C. beaquaertii* and *C. mossii*; Fig. 3) using DEC+J focusing on **Afrotropical regions and using a distance matrix** (orange = Palearctic; light blue = Ethiopia, dark blue =Tropical East Africa; pink = Madagascar; purple =Southern Africa).

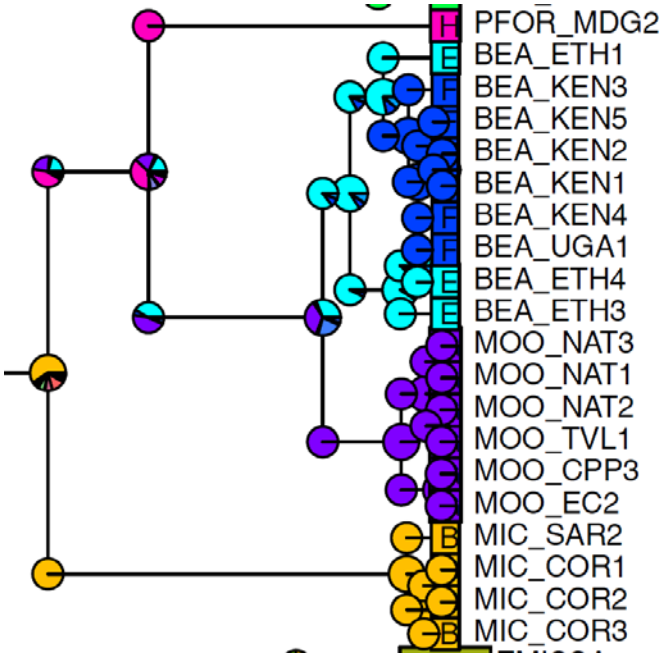


Figure S13.

Results of the BioGeoBEARS analysis for the complete dataset using **DEC+J only coding Palearctic** regions (A [dark blue] = Northern Europe: Denmark, Norway, Sweden; B [light blue] = Western Europe: Belgium, Central and Northern France, British Iles, Netherlands; C [green] = Southern Europe and North-western Africa: Portugal, Spain, Southern France including Corsica, Italy including Elba and Sardinia, Southern Switzerland, as well as Morocco, Macaronesian Islands of Azores and Madeira; D [yellow] = South-eastern Europe: Greece, Cyprus, western Turkey as well as Croatia and Serbia, E [orange] = Central Europe: Austria, Czech Republic, Germany, Hungary, Slovakia, Slovenia, Northern Switzerland; F [red] = Western Asia (Azerbaijan, Eastern Turkey, Georgia, Iran), G [pink] = Sub-Saharan Africa and Madagascar). Maximal number of areas allow 7.

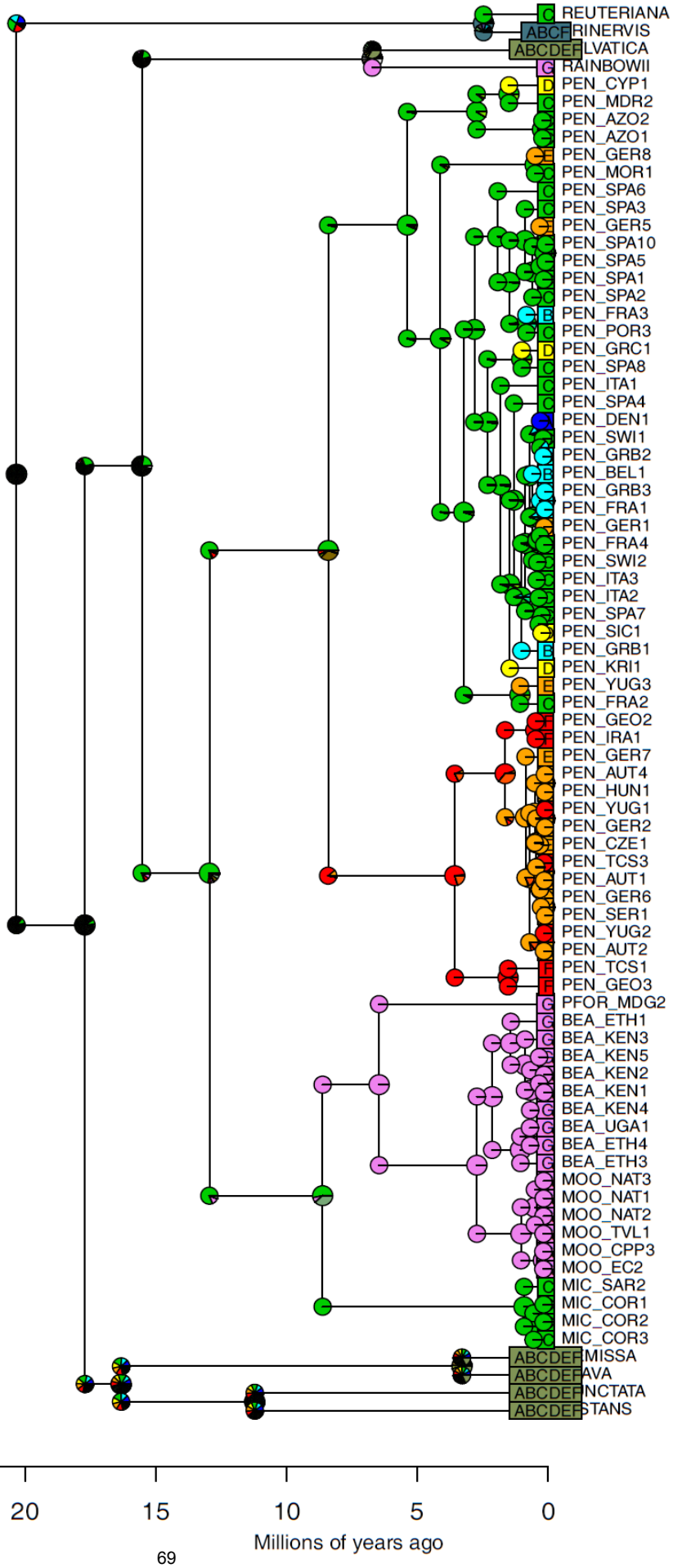
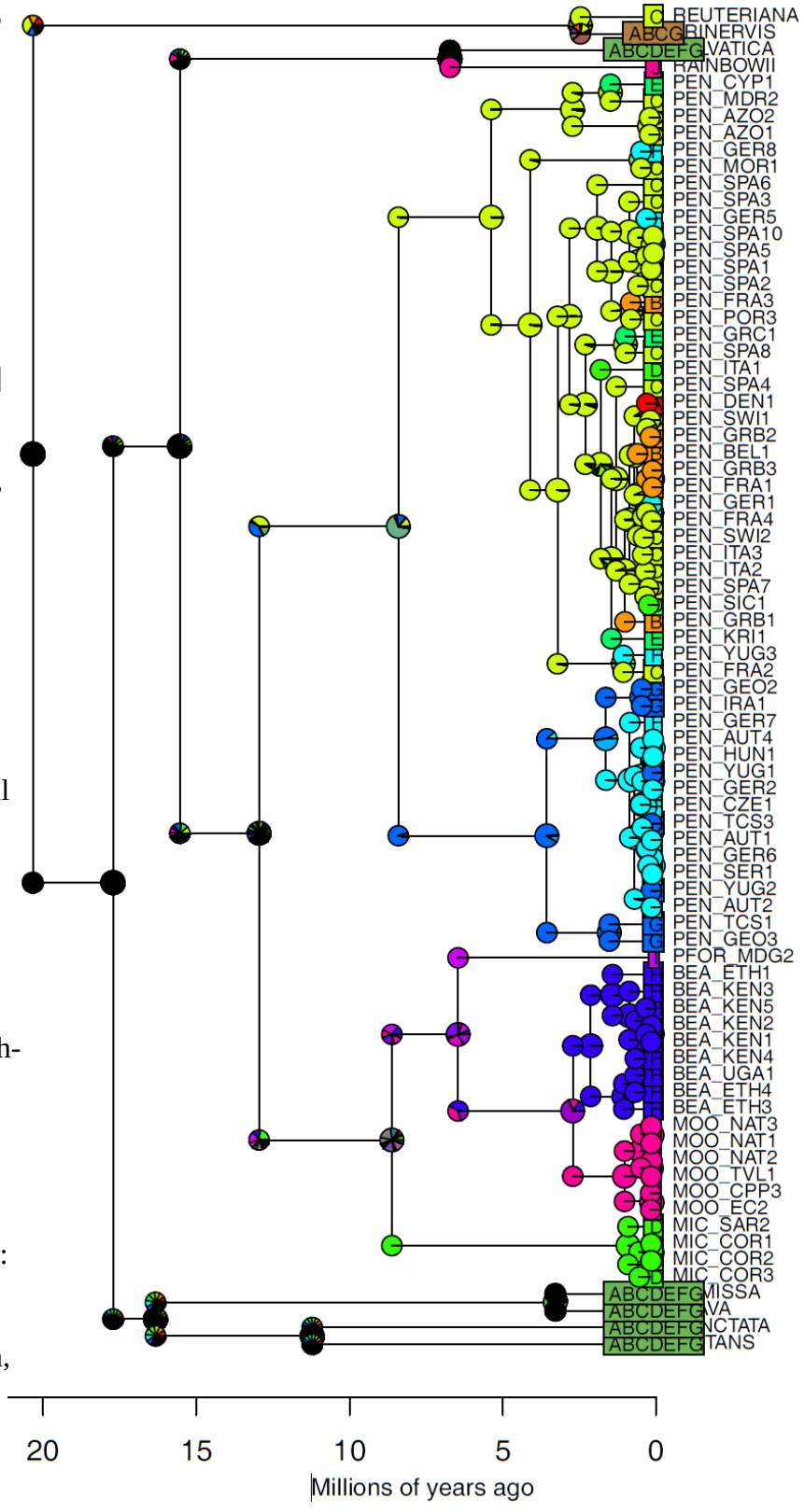


Figure S14.

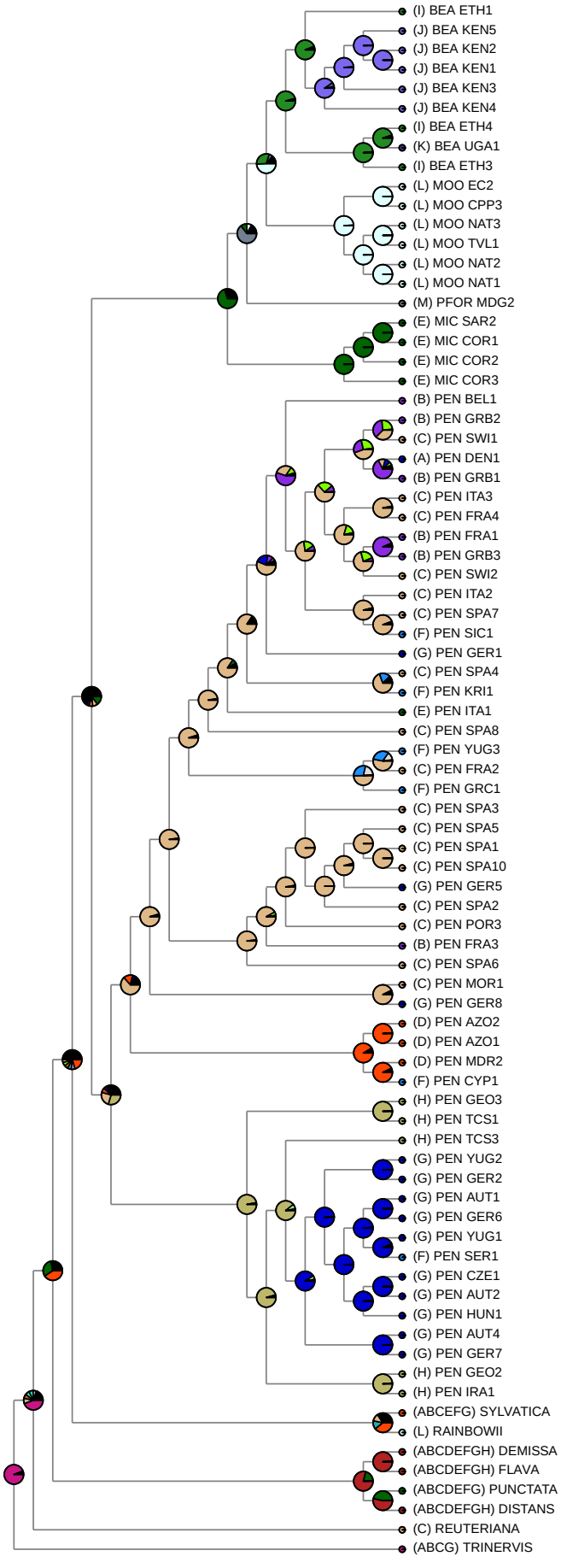


Result of the BioGeoBEARS analysis for the complete dataset using **DIVA-Like+J coding Palearctic** regions with **separate Thyrrhenian Sea Islands** (A [red] = Northern Europe: Denmark, Norway, Sweden; B [orange] = Western Europe: Belgium, Central and Northern France, British Isles, Netherlands; C [light green] = Southern Europe and North-western Africa: Portugal, Spain, Southern France, Italy, Southern Switzerland, as well as Morocco, Macaronesian Islands of Azores and Madeira; D [bright green] = Corsica, Elba and Sardinia Sicily; E [blue green] = South-eastern Europe: Greece, Cyprus, western Turkey as well as Croatia and Serbia, F [light blue] = Central Europe: Austria, Czech Republic, Germany, Hungary, Slovakia, Slovenia, Northern Switzerland; G [blue] = Western Asia (Azerbaijan, Eastern Turkey, Georgia, Iran), H [dark blue] = East Africa and Ethiopia; I [purple]

= Madagascar. J [pink] = Southern Africa. Circles that appear black have too many possible state to separate clearly. Maximal number of areas allowed 7.

Figure S15.

Result of the BBMA analysis for the complete dataset using **DIVA_LIKE+J only coding** **Palearctic** regions (A [dark blue] = Northern Europe: Denmark, Norway, Sweden; B [purple] = Western Europe: Belgium, Central and Northern France, British Isles, Netherlands; C [ocra] = Southern Europe and North-western Africa: Portugal, Spain, Southern France including, Italy, Southern Switzerland, as well as Morocco; D [light red] = Macaronesian Islands of Azores and Madeira; E [dark green] = Thyrrhenian Sea islands = Corsica, Elba and Sardinia; F [light blue] = South-eastern Europe: Greece, Cyprus, western Turkey as well as Croatia and Serbia; G [dark blue] = Central Europe: Austria, Czech Republic, Germany, Hungary, Slovakia, Slovenia, Northern Switzerland; H [light brown] = Western Asia (Azerbaijan, Eastern Turkey, Georgia, Iran); I [green] = Ethiopia; J [violet] = Kenya; K [grey] = Uganda; L [light blue] = Southern Africa; M [light grey] = Madagascar).



Chapter 3. Typification of 18th Century names in *Carex* sect. *Rhynchocystis* (Cyperaceae): *Carex pendula* and allies

Typification of 18th Century names in *Carex* sect. *Rhynchocystis* (Cyperaceae): *Carex pendula* and allies

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Abstract

According to recent molecular and morphological studies, the apparently homogeneous *Carex pendula* Huds. has been revealed to include two different overlooked species. One species is distributed across Western Europe and the Mediterranean, whereas the other ranges from Central and Eastern Europe to the Caucasus and northern Iran. Three different names have been considered synonyms of *C. pendula*: *C. agastachys* L.f., *C. maxima* Scop., and *C. mutabilis* Willd. Our unexpected finding raised the question of what name should apply to each taxon. We found *Carex pendula* to apply to the western taxon, and *Carex agastachys* to be the prior name for the eastern one. We perform the typification ofthe names when necessary. We also designate an epitype for *C. pendula* given the insufficient level of detail provided by the previously designated neotype.

Key words: epitypification, Hudson, lectotypification, Linnaeus, Scopoli, Willdenow

Introduction

Carex pendula Huds. was described in *Flora Anglica* (Hudson, 1762). In the protologue, Hudson cited in synonymy three previously published polynomial names. The first, from Parkinson (1640), was also cited from Ray (1724). The second, from Bauhin (1620), was also cited from Bauhin (1623) and Bauhin & Cherler (1651). Lastly, the third was cited from Ray (1724). Unfortunately, there seems to be no existent voucher material that can be associated with either Hudson's name, or the cited polynomials. Egorova (1999) neotypified *C. pendula* Huds. using a Morison (1699) plate, as it was also cited by Hudson, but in the later 2nd and 3rd editions of *Flora Anglica* (Hudson, 1778, 1798).

Míguez & al. (tentatively accepted, and in prep.) have recently found, based on molecular and morphological data, that the apparently homogeneous *C. pendula* actually includes two overlooked, mostly allopatric, taxa. One taxon is distributed across Western Europe (eastward to Germany) and the Mediterranean (including Greece and Cyprus). The other ranges from Central and Eastern Europe to the Caucasus and northern Iran. There seems to be an uncertainly delimited contact zone between both taxa in Central Europe across Germany, Austria and the former Yugoslavia. According to Míguez & al. (in prep.), the species are readily distinguished based on several morphological characters, among others, including the ligules (with whitish borders in the western taxon vs. reddish in the eastern one), female spike

peduncles (smooth vs. scabrid), and achenes (elliptic vs. obovate). The unexpected finding of these two clearly delimited taxa raises the question of what name should apply to each of them.

Carex pendula was described from the outskirts of London ("[…] inter Hampstead et Highgate […]"; […between Hampstead and Highgate…]; Hudson, 1762). Thus, it seems clear that the name *C. pendula* Huds. should apply to the western taxon. Egorova's (1999) typification agrees with this view, as Morison's (1699) work also refers to English plants. However, the illustration that Egorova chose shows an insufficient level of detail to allow the critical characters that distinguish the two taxa to be observed. Accordingly, under the International Code of Nomenclature for algae, fungi, and plants (ICN hereafter), Art. 9.8 (McNeill & al. 2012), we are here designating an epitype from the vicinity of the type locality (see below) which unambiguously belongs to the western taxon. In addition, a sample of this epitype has been included in a molecular study, the results of which confirm that the specimen is identifiable with the western taxon (Míguez & al., tentatively accepted).

Regarding the name that should apply to the eastern taxon, we proceeded to check the identity of three additional names, also described in the late 18th century, that are traditionally referred to the synonymy of *C. pendula* Huds. (by e.g. Chater, 1980; Jiménez-Mejías & Luceño, 2011; Govaerts & al., 2016): *Carex maxima* Scop. (1772), *C. agastachys* L.f. (1782), and *C. mutabilis* Willd. (1787).

Carex maxima Scop. was reported as a plant from Carniola (currently Slovenia; Scopoli 1772), an area where the occurrence of both species seems to be possible (Míguez &

al., in prep.). No specimen was explicitly cited in the protologue (Scopoli, 1772). However, a voucher from Scopoli's herbarium housed at LINN (LINN-HL1100.94) revealed characters that agree with *C. pendula* Huds. (female spike peduncles smooth, and [bract] ligules whitish). This specimen carries a label, in Scopoli's hand, bearing the name "*Carex maxima*", so confirming that it corresponds with Scopoli's own concept of this name. Consequently, we are designating this specimen as the lectotype of *Carex maxima* Scop., consolidating this name as a synonym of *C. pendula* Huds. *s.s*. *Carex agastachys* L.f. was published on the basis of materials from the *Phytophylacium Ehrhartianum exsiccatae*, specifically number 19 (Linnaeus, f., 1782). The locality where this material was collected (Hannover, Germany) lies in the still poorly known contact area between *C. pendula* s.s. and the eastern taxon (Míguez & al., tentatively accepted, and in prep.). Specimens belonging to Ehrhart's exsiccatae have been found at M, GOET, and LINN herbaria. The detailed study of the specimen housed at M readily revealed characters unequivocally associated with the eastern taxon (female spike peduncles conspicuously scabrid and [bract] ligules reddish). We designate the voucher at LINN (LINN-HS1441-174-1), which was almost certainly studied by Linnaeus f., as lectotype of *Carex agastachys* L.f. (see ICN, Rec. 9.A.1; McNeill & al. 2012), which becomes the correct name to be applied to the *C. pendula*-like eastern taxon.

Subsequently, the name *Carex mutabilis* Willd. (Willdenow, 1787) was described based on plants from Berlin area ([…]prope Spandau hinter den Schülerbergen[…]; […]near Spandau, behind the Schülerbergen[…]). The protologue was accompanied by

the figures of two varieties, α & β (Willdenow, 1787: Tab. II, Figs. 6 & 7, respectively), neither of them validly published. Whereas variety α must be a *C. pendula*-like plant according to its origin (NE Germany) and description (i.e. “stigma trifidum”; among other characters). However, the drawing lacks the characters that allow discrimination between the western and eastern taxa. variety β is not unequivocally identifiable,

We examined materials from Willdenow’s herbarium at the Digital Herbarium of Berlin (Röpert, 2000–2016) but there is no specimen that can unequivocally be associated with the protologue nor with the plant pictured in the figure. On the other hand, all the materials in the Willdenow´s herbarium at B listed under *C. pendula* display reddish ligule borders on the bract, a character that matches the eastern taxon. Moreover, the voucher B-W-17248 (specifically the stem at the left) has the lowermost spike with a conspicuously scabrid peduncle. Given the absence of any better material, we here lectotypify *C. mutabilis* Willd. using the figure provided in the protologue, and epitypify (ICN, Art. 9.8, McNeill & al., 2012) the name using the above-cited voucher from Willdenow’s herbarium. Our decision to typify the name *C. mutabilis* Willd. using the plate depicting variety α respects the current usage of the name as a *C. pendula*-like plant (ICN, Rec. 9.A.4). Thus, *C. mutabilis* Willd. should be removed from the synonymy of *C. pendula s.s.* and instead be considered a synonym of *C. agastachys*.

Carex pendula Huds., Fl. Angl.: 352 (1762) - Ind. loc.: “Habitat in sylvis et sepibus humidis; in sepibus inter Hampstead et Highgate copiose.” - Neotype designated

by Egorova (1999): Morison, 1699, Pl. Hist. Univ, 3, sect. 8, tab. 12, fig. 4. - **Epitype (designated here, supporting the neotype cited above)**: "London. Hampstead Heath, between Hampstead and Highgate, Kenwood lake –vc 21, Middlesex, 51.568527 N 0.166402 W, M. A. Spencer MAS-2012-040, 5 Aug 2012" (BM001074530!; iso- UPOS-5004!).

= *Carex maxima* Scop., Fl. Carniol., ed. 2, 2: 229 (1772) – Ind. loc.: not explicit [Carniola, Slovenia] - **Lectotype (designated here)**: G.A. Scopoli, s.n. (LINN-HL1100-94!).

Carex agastachys L.f., Suppl. Pl.: 414 (1782) - Ind. loc.: "Habitat in Germania" . - **Lectotype (designated here)**: "19 Agastachys. *Carex agastachys* L., Hannoverae, F. Ehrhart" [Phytophylacium ehrhartianum] (LINN-HS1441-174-1, photo!; iso- GOET-002819!, M sn!).

= *Carex mutabilis* Willd., Fl. Berol. Prodr.: 37 (1787). - Ind. loc.: "In pratis & fossis prope Spandau hinter den Schülerbergen rarius" - **Lectotype (designated here)**: Willdenow, 1787, Fl. Berol. Prodr., Tab. II, Fig. 6. - **Epitype (designated here, supporting the lectotype cited above)**: B-W-17248 (Willdenow's herbarium at B, photo!)

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Chapter 4. Good practice to the rescue of taxonomy: overlooked species and overvalued traits in the giant sedges of *Carex* section *Rhynchocystis* (Cyperaceae)

Good practice to the rescue of taxonomy: overlooked species and overvalued traits in the giant sedges of *Carex* section *Rhynchocystis* (Cyperaceae)

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Abstract

Carex section *Rhyncocystis* is currently composed of five species of giant sedges distributed between the Western Palearctic (*C. microcarpa* and *C. pendula*) and sub-Saharan Africa (*C. bequaertii, C. mossii, C. penduliformis*). Recent phylogenetic studies revealed that *C. bequaertii* and *C. mossii* are not mutually monophyletic, and that *C. pendula*, despite monophyletic, actually embraces two divergent lineages. In order to objectively assess the taxonomic circumscription of the species in section *Rhynchocystis* we performed a morphometric study based on herbarium vouchers. Our analyses revealed clear-cut diagnostic characters neglected by the traditional taxonomy of the group, whereas we found that other apparent traits were overvalued and not diagnostic. Our results suggest five species. Only *C. microcarpa* and *C. penduliformis* were supported in their traditional concepts. The two *C. pendula* lineages corresponded to two morphologically distinct, mostly allopatric taxa: *C. pendula* s.s. in Western Europe and the Mediterranean, and *C. agastachys* in Central and Eastern Europe and Southwestern Asia. On the contrary, *C. bequaertii* and *C. mossii* were found to be weakly morphologically differentiated and thus better treated as a single species with two disjunctly distributed subspecies. Our study shows how the good practice in taxonomy lead to robust and even unexpected results in groups of plants supposedly well-studied.

KEYWORDS: cryptic species – morphometrics – Mann–Whitney U test – micromorphology – multivariate analyses – principal component analysis.

INTRODUCTION

Traditional classification of *Carex* L, as for most plants, has largely relied on morphological characters, especially those related to reproductive structures (Kükenthal, 1909; Chater, 1980; Egorova, 1999). Misconception about which characters are diagnostic and which are not is often the main problem underlying the incongruence between morphology-based classifications and the phylogenetic relationships revealed by DNA sequences (Global *Carex* Group, 2016). These conflicts have been evidenced in several sections of *Carex* (Roalson, Columbus, & Friar, 2001; Hipp et al., 2006; Maguilla et al., 2016; Molina, Chung, & Hipp, 2015; Gebauer, Roeser, & Hoffmann, 2015). The identification of synapomorphic combinations of diagnostic characters in sedges is frequently problematic, mainly because reproductive structures in *Carex* are morphologically reduced and, as consequence, species may look superficially similar (González-Elizondo & Peterson, 1997; Starr, Harris, & Simpson, 2004). In addition, showy homoplasic features may contribute to obscure the taxonomy in plants groups with reduced morphologies, as they are more evident than more subtle synapomorphies (e.g. Jiménez-Mejías *et al.*, 2013; Otero *et al.*, 2014; Ronikier & Zalewska-Gałosz, 2014). This unbalance between overvalued homoplasic characters and neglected synapomorphic ones is, in many cases, the responsible for the disagreements between morphologically-based taxonomic classification and DNA-based phylogenies (Naczi, 2009; Jiménez-Mejías & Vargas, 2015; Global *Carex* Group, 2016). A particular case of taxonomic conflict are the so-called cryptic species species that are apparently morphologically indistinguishable, frequently united under a single Linnean name in traditional classifications, until molecular analyses tell them apart (Bickford et al., 2006).

Taxonomic treatments of *Carex* sect. *Rhynchocystis* Dumort have remained fairly stable throughout the history with little controversy in species delimitation (Kükenthal, 1909; Chater, 1980; Egorova, 1999). The section was recently narrowed to embrace only the five Western Palearctic and Afrotropical species (see Table 1). As newly delimited, the group is remarkably morphologically well-defined throughout its entire range from North Europe to the Cape Region (Nelmes, 1940; Gehrke, 2011). *Carex* sect. *Rhynchocystis* taxa are distinguished by: 1- being large densely cespitose perennial plants, with stems and leaves usually around 1 m or even more; 2- having racemose inflorescences, with proximal long-sheathing leaf-like bracts, the blade surpassing the inflorescence; and 3- displaying elongated cylindrical, densely-flowered, and usually flexuous spikes, the lowermost ones often pendent (Kükenthal, 1909; Egorova, 1999).

Recent phylogenetic analyses (Míguez et al., in press) showed that relationships among lineages retrieved using molecular analyses were not entirely congruent with taxonomic treatments of *Carex* sect. *Rhynchocystis* based on morphological characters. Molecular evidence suggests that the section constituted a well-supported monophyletic group arranged in two major linages. The first lineage includes the Mediterranean *C. microcarpa* Bertol. Ex Moris as sister to the African species *C. bequaertii* De Wild, *C. mossii* Nelmes, and *C. penduliformis* Cherm. *Carex microcarpa* and *C. penduliformis* were found to be monophyletic, whereas *C. bequaertii* and *C. mossii* were not mutually monophyletic. The second main lineage comprised the taxon called to date *C. pendula* Huds, widespread in the western Palearctic. However, despite being monophyletic, *C. pendula* was found to include two highly differentiated genetic lineages, with an ancient divergence dating back to the Miocene (Míguez et al., in press). These two lineages were mostly allopatric, with an eastern lineage distributed from central Europe to the

Caucasus and western Iran, and a western lineage inhabiting Western Europe east to Germany, the Mediterranean Basin (including northwestern Africa and southwestern Asia), and Macaronesia (Míguez et al.,in press). The lack of mutual monophyly of *C. bequaertii* and *C. mossii*, as well as the deep split between *C. pendula* lineages poses an incongruence between the phylogenetic structure of the section, and the morphology-based scheme (Table 1).

Multivariate statistical analysis methods based on large sets of morphological data have been long and widely used in the search of taxonomic significance as morphology-based predictors for species delimitation. These analyses help to detect morphological discontinuities between taxa and to identify diagnostic characters (see Crins & Ball, 1988; Valcárcel & Vargas, 2010; Jiménez-Mejías, Luceño, & Martín-Bravo, 2014; Więcław *et al.*, 2016). In contrast with treatments based in "taxonomical expertise", which might be subjective, these techniques standardize the measure of morphological variability and objectively quantify the differences among groups (Valcárcel & Vargas, 2010).

The inclusion of micromorphological and anatomical characters have sometimes been demonstrated to support taxonomic partitions based on macromorphological characters in sedges (Naczi, 2009). Scanning electron microscopy (SEM), for example, has been used to observe some microstructures of the achene epidermis in *Carex* and have sometimes been shown to exhibit significant interspecific variation for some groups of closely related species (Starr & Ford, 2001; Zhang, 2006; Naczi, 2009; da Silva et al., 2011).

The integration of molecular and morphological data has contributed to re-evaluate taxonomic relationships of other plant groups within an updated evolutionary context (Martín-Bravo & Jiménez-Mejías, 2009; Jiménez-Mejías et al., 2012;

Fernández-Mazuecos et al., 2014; Vigalondo et al., 2016). We performed a morphometric study as part of our comprehensive systematic revision of the *Carex* sect. *Rhynchocystis*. Our main aim is to integrate our previous DNA-based phylogenetic results with the quantitative and qualitative morphological variation observed, using a rigorous, statistically-based objective framework. The particular objectives are to (1) reevaluate the morphological affinities between the five traditionally considered species (2) assess if the genetic differentiation between the two lineages in *C. pendula* s.l. is correlated with morphological differences; (3) evaluate the taxonomic status of the phylogenetically poorly differentiated *C. bequaertii* and *C. mossii*; and (4) provide a revised taxonomic treatment for the whole section.

MATERIALS AND METHODS

Study group and sampling

For the sake of simplicity, we follow an updated taxonomic treatment based on our previous phylogenetic results (Míguez et al. in press) that includes: *C. bequaertii*, *C. microcarpa*, *C. mossii*, *C. pendula* and *C. penduliformis*, plus *C. agastachys* (Table 1) forthe eastern lineage of *C. pendula* s.l. (Míguez et al.,in press ; Jiménez-Mejías et al., in press). Conversely, our concept of *C. pendula* only refers to the western lineage.

We studied 245 herbarium specimens (Appendix 1) from the following 19 herbarium collections: B, E, K, LISU, M, MADJ, MHA, P, PRE, TUM, UPOS, UPS, Z (abbreviations according Index Herbariorum; Thiers 2016). We measured 109 specimens in total: 24 specimens of *C. agastachys*, 21 of *C. bequaertii*, nine of *C. microcarpa*, 17 of *C. mossii*, 35 of *C. pendula*, and three of *C. penduliformis***.** A special effort was made to represent the distribution range and morphological variability of each taxon. In addition we studied 16 specimens from HUB, KRA, Koopman (personal

Herbarium), MA, MTMG, NY, and SS herbaria to complement the distributions and ecology of the different taxa in our treatments. Unfortunately the sampling of *C. penduliformis* and *C. microcarpa* was strongly limited due to their scarce representation in herbaria. The distribution of taxa was revised, using TDWG geographical codes at level 3 ('Botanical countries') for specifying the ranges (Brummit, 2001).

Morphological characters and measurements

We selected, 25 quantitative continuous, four quantitative discrete, and four qualitative, potentially diagnostic characters (Table 2) based on previous taxonomic accounts of the group (Kükenthal, 1909; Nelmes, 1940; Chater, 1980; Haines & Lye, 1983; Gordon-Gray, 1995; Egorova, 1999; Gehrke, 2011), and our own observations. Measurements were taken using an ocular micrometer, with the exception of the largest characters (more than 10 mm), which were measured using a standard 30-cm rule. The number of prickles on female spike peduncle was counted on the 0.5 cm distal portion of the peduncles of the proximal and distal female spike. Two or three mature stems were measured per specimen and their averages included in the analyses. Qualitative characters were codified according to states reported in previous *Carex* treatments (Kükenthal, 1909; Chater, 1980; Egorova, 1999) and personal observations (Table 2).

Statistical analyses

Three different types of multivariate statistical analyses were carried out. Principal components Analysis (PCA) and Discriminant Function Analysis (DFA) were used to identify and test morphogroups. As most of our data did not meet the assumption of normality, we used the Mann-Whitney U test to statistically validate the most discriminant characters differentiating between pairs of the species. The level of

significance was set at $P < 0.01$ and run in R (http://www.r-project.org/).). All statistical analyses were performed using the software IBM SPSS statistics v.22 (Chicago, Illinois, USA). Our analytical approach is inspired by the hierarchical procedure presented in Valcárcel and Vargas (2010) for *Hedera*, and applied in *Carex* by Jiménez-Mejías, Luceño, & Martín-Bravo (2014).

Principal Component Analyses (PCA) — We performed consecutive PCAs (Fig. 1). We started with a PCA including all the samples. To achieve the best split among morphogroups we performed a first exploratory PCA with the 32 variables, retaining later only those 1- with the highest principal component (PC) loadings, and 2- with the higher correlation coefficients whenever the correlation between characters was found not to be redundant (characters purge; Table 2). When the analysis identified morphogroups, i.e., separate clusters containing more than one species, the samples on these morphogroups were split as a new subset and subsequently reanalyzed separately, including again all the characters and performing a new purge. Due to the lack of male spikes in *C. mossii* and *C. bequaertti*, INFLM and INFWM variables (Table 2) were discarded in the analysis in which these species were included. Kaiser´s measure of sampling adequacy and Barlett´s test of sphericity were performed to evaluate the suitability of the data (Valcárcel & Vargas, 2010; Jiménez-Mejias et al. 2014). Only principal components with Eigen-values greater than 1 were retained.

A first PCA (PCA-I) was performed using 13 variables and the 109 specimens to explore the complete dataset (*C. agastachys, C. bequaertii, C. microcarpa, C. mossii, C. pendula* and *C. pendulifomis*) (see Table 2). This PCA split the dataset into two main clusters. New PCAs were performed for each of these subsets (PCA-II and PCA-V, respectively; Fig. 1) PCA-II was performed on subset 1, containing *C. agastachys, C. microcarpa, C. pendula* and *C. penduliformis*, six variables and 69 specimens. PCA-V

was performed on subset 2, containing *C. bequaertii* and *C. mossii*, analyzing 13 variables and 39 specimens.

Two new subsets were obtained from the PCA-II of subset 1, split and reanalyzed (Figs. 1, 2; Table 2): PC-III was conducted on subset 1.A, containing *C. agastachys* and *C. penduliformis*, and analyzing 13 variables and 28 specimens, and PCA–IV was performed on subset 1.B, containing *C. microcarpa* and *C. pendula*, and including 13 variables and 43 specimens (Figs. 1, 2; Table 2).

The final morphogroups groups detected at the end of each chain of consecutive PCAs were considered indicative of morphological distinctiveness within the known phylogenetic framework of the section. Accordingly, we refer to these groups as homogeneous morphogroups.

Discriminant Function Analysis (DFA) — After the identification of homogeneous morphogroups, discriminant function analysis (DFA) was used to test the taxonomic significance of both the morphogroups and our a priori delimited 5-species taxonomic treatment as described in Valcárcel & Vargas (2010). We randomly selected 70% of all samples to perform the DFA using a cross-validation of the model over these samples. Then, the remaining 30% of the samples were randomly excluded from the analyses and used as a confirmatory blind control. We considered as significant those groups that correctly classified >80% of the excluded cases. DFA-I was performed over the entire dataset using all the 28 quantitative variables and the 5 morphogroups corresponding with the species *C. agastachys*, *C. microcarpa*, *C. pendula*, *C. pendulifomis* and the homogeneous morphogroup formed by the subset 2 (*C. bequaertii* and *C. mossii*). Due to the absence of a clear split between *C. bequaertii* and *C. mossii* in PCA-V (see Results), both taxa were treated in this analysis as a single homogeneous

morphogroup. The variables INFLM and INFWM were discarded because *C. mossii* and *C. bequaertii* do not have male spikes.

DFA-II was performed to find taxonomic significance between the homogeneous morphogroup of subset 2 (*C. bequaertii* and *C. mossii*). We used the same 13 variables than in the PCA-V (Table 2).

Micromorphological study

Micromorphology of the achene was examined under a Scanning Electron Microscopy (SEM; GeminiSEM 300, Zeiss, Germany). One representative achene per considered specimen was examined. The achenes epidermis were studied in detail to evaluate if there were interspecific variation in silica bodies between the different taxa. Achenes were treated to remove the anticlinal and outer periclinal walls of the epidermis cells, in order to allow the observation of silica bodies that are placed on the inner anticlinal walls. Our procedure was modified from Salo *et al*. (1994). Achenes were digested in a solution of acetic anhydride and sulphuric acid (9:1) for 24 hours at room temperature, washed with distilled water, and then placed in an ultrasonic bath in a Ultrasonic cleaner (Branson 2510E-MT) for 10 minutes. Finally, achenes were air-dried at room temperature on Petri dishes. When the anticlinal and outer periclinal walls were not totally removed by this treatment, we repeated the treatment with new achenes but increasing the time of ultrasonic bath to 20 minutes. Prior to observation under SEM, the achenes were gold-coated.

Two pictures with different zooms were taken from each sample: an image of the entire achenes to visualize their shapes (Fig.3-A, C, E, G, I, K), and a second picture showing the micromorphological features of the achenes in detail (Fig.3-B, D, F, H, J, L).

RESULTS

Statistical analyses

In all datasets Kaiser´s measure of sampling adequacy was >0.5, and Barlett´s test of sphericity was significant. This implies that the sampling sizes were suitable to be explored using PCA (cf. SPSS 2009; Valcárcel & Vargas, 2010; Jiménez-Mejías et al., 2014). Principal components extracted in each PCA are referred as PC and numbered using roman numerals.

Principal Component Analysis (PCA)

PCA-I —We extracted three principal components (PCs) that accounted for 66.4% of the total variance (42.67%, 14.71%, and 9.02% respectively). The scatter-plot PC-1 vs PC-2 revealed a general structure with two major morphogroups (Fig. 2-A). An even better separation between the two morphogroups was obtained in the scatter-plot PC-1 vs PC-3 (Appendix 2-A). One morphogroup included *C. pendula*, *C. microcarpa*, *C. penduliformis,* and *C. agastachys*. The second morphogroup included the African species *C. mossii* and *C. bequaertii*. The characters that contributed the most to the first two components were SUS, PSCLL, SPKMN and DSFS (Table 2).

PCA-II—We extracted two PCs explaining 75.5 % of the total variance (48.90% and 26.58% respectively) (Fig. 2-B). PCA-II revealed a general underlying structure with two morphogroups, one including the samples belonging to *C. agastachys* and *C. penduliformis*, and another that included those of *C. pendula* and *C. microcarpa*. The characters that contributed the most to the first two components were PLC, ACHL, UL and DLC (Table 2).

PC-III—We extracted four PCs explaining 73.7% of the total variance (29.41%, 26.24%, 10.14%, and 7.94% respectively) (Fig. 2-C. Appendix 2-B, C). The scatter-plot PC-1 vs PC-2 of the PCA-III revealed a general structure with two morphogroups

clearly separating *C. agastachys* and *C. penduliformi*s (Fig. 2-C). This separation was not found in the scatter-plots PC-1 vs PC-3 or PC-1 vs PC-4 (Appendix 2- B, C). The characters that contributed the most to the first two components were UBL, SSCLW, INFWF (Table 2).

PC-IV— We extracted three PCs explaining 67% of the total variance (41.41%, 16.37% and 9.12 % respectively). The scatter-plot PC-1 vs PC-2 of the PCA-IV revealed a general structure with two morphogroups separating *C. microcarpa* and *C. pendula* (Fig. 2-D). The scatter-plot PC-1 vs PC-3 of the PCA-IV also showed a scarce overlap between the two species (Appendix 1-D). The characters that contributed the most to the first two components were LUMWD, DLC and UPLMS (Table 2).

PCA-V— We extracted four PCs explaining 68.48% of the total variance (26.05%, 18.25%, 14.05% and 10.13% respectively)**.** In all scatterplots, PC-1 vs PC-2, PC-1 vs PC-3 or PC-1 vs PC-4, *C. mossii* and *C. bequaertii* remained intermingled, and no clear separation of the samples of each species as a homogeneous morphogroup was obtained (Fig. 2-E, Appendix 1-E, F).

Discriminant Function Analysis (DFA)

DFA-I — The variables with the highest discriminant scores for the five considered homogeneous morphogroups were SSCLL, ACHL, SPKMN, UL, ACL, USL, INFLF, LUMWD (Appendix 3). Validation using excluded cases significantly supported a clear distinction among *C. agastachys*, *C. microcarpa*, *C. pendula*, *C. pendulifomis* and the homogeneous morphogroup formed by *C. bequaertii*-*C. mossii*. Thus, a high correspondence between the identified homogeneous morphogroups and classifications resulting from DFA (Wilks´lambda =0-0.3; p=0) was retrieved, correctly classifying 97.2% of unselected original grouped cases. All morphogroups were classified correctly

at 100% for unselected cases, excepting *C. pendula* that was correctly classified at 93.3% (Appendix 3).

DFA-II— The highest scores for the discriminating between *C. bequaertii* and *C. mossii* were obtained by the characters ACHW, UPLDS, DLC (Appendix 3). However, validation using excluded cases was not significant for the discrimination of *C. bequaertii* from *C. mossii*, as only 69.2% of all the excluded cases were correctly classified (Wilks´lambda=0.237; p=0.033). In particular, 71.4% of all *C. bequaertti* unselected cases were correctly classified, and 66.7% were for *C. mossii* (Appendix 3). These values did not meet the levels of significance considered in this study (80%), further supporting the consideration of both species as a single homogeneous morphogroup.

Mann-Whitney U test

Despite some overlap in the range of many characters between morphogroups, Mann-Whitney U test retrieved significant differences (P-value<0.01) between morphogroups for a number of variables (Table 2). Between 2 and 20 characters were found to be significantly different (P-value <0.01) among all pairwise comparisons at homogeneous morphogroup level (Table 3).

Boxplots and histograms

Boxplots and histograms of the most discriminant characters retrieved by DFA or with less than 25% overlap are shown in Figs. 4 and 5 respectively. Only subtle differences in Achene width (ACHW) and Maximum length of the pistillate scale colored margin (MAXCLPM) were detected between *C. bequaertii* and *C. mossii*.

Micromorphological study

The differences in the achene shape among species revealed by the macromorphological study were evident under SEM microscopy imaging (Fig. 3-A, C, E G, I, K); The

achenes of all species in section *Rhynchocystis* were trigonous in cross section, in most cases constricted proximally into a substipitate base, and epapillose. *Carex agastachys* and *C. microcarpa* displayed achenes obovate, with the widest point near the top, whereas *Carex pendula* presented achenes elliptic, with the maximum width at the middle or slightly above it. The samples of *C bequaertii* and *C. mossi* had achenes ranging from obovate to elliptical. Eventually, *C. penduliformis* achenes ranged from obovoid to ± ellipsoid. The micromorphology of the epidermic cells was similar in all the studied samples and no differences for any of the species were evident. The shape of the epidermis cells are polygonal and the anticlinal wall straight in all species (Fig. 3-B, D, F, H, J, L). The silica platforms on the inner periclinal wall were flat or very slightly concave for all species (Fig. 3-B, D, F, H, J, L). Most of the time only one large central silica body was observed within each cell. In addition, a few sparse cells of *C. bequaertii* and *C. mossi* were found bearing two smaller bodies instead (Fig. 3-B, D, F, H, J, L). No smaller satellite silica bodies or pits were found (Fig. 3-B, D, F, H, J, L).

DISCUSSION

Overlooked species and overvalued characters revealed by objective statistical analyses.

Previous phylogenetic reconstructions in *Carex* have revealed that there are incongruences between previously proposed morphology-based classifications and the DNA-based phylogenies (Roalson *et al.*, 2001; Hipp *et al.*, 2006; Escudero & Luceño, 2009; Dragon & Barrington, 2009; Molina *et al.*, 2015; Global Carex Group, 2016). The main reason behind these incongruences is that morphological characters traditionally used to diagnose groups and species in *Carex* are frequently homoplastic (Hipp *et al.*, 2006; Molina *et al.*, 2015; Global Carex Group, 2016). Due to the frequent absence of

single morphological synapomorphies, a combination of characters is usually needed to define sections in *Carex* (Hipp *et al.*, 2006; Gebauer, Roeser, & Hoffmann, 2015; Molina *et al.*, 2015; Global Carex Group, 2016). This is the case of *Carex* sect. *Rhynchocystis*, which is a morphologically cohesive and easily recognizable group, well-defined by a combination of characters.

The examination of morphological variation in *Carex* sect. *Rhynchocystis* using our multivariate analyses and statistical validations revealed that, while some characters traditionally reported as diagnostic in *Carex* sect. *Rhynchocystis* (e.g. utricle size or pistillate scale length; Nelmes, 1940; Gehrke, 2011) showed wide overlap (Figs 4, 5). Others like achene shape or ligule color are good species-distinguishing diagnostic characters not previously considered. Our study exemplifies how traditional treatments might overweight certain characters, while those truly diagnostic remains unnoticed. The same pattern has been found in previous works combining statistical morphological and molecular analyses that resulted in novel taxonomic recircumscriptions (e.g. *Carex viridistellata* Derieg *et al.*, 2013), *Utricularia* sect. *Foliosa* (Lentibulariaceae) (Baleeiro, Jobson, & Sano, 2015), *Carex furva* s.l. (Maguilla *et al.*, 2016), *Carex sylvatica* s.l. (Benítez-Benítez *et al*., accepted). The power of these combined approach is remarkable in our case when considering that one of the taxa that we re-define is *C. pendula*, a quite large plants, and fairly common through a so well-studied area as Europe.

Contrasting patterns of morphological and phylogenetic relationships

Our study helps to re-define taxonomic groups within *Carex* sect *Rhynchocystis* in accordance with its phylogeny. Five morphologically well-defined species should be recognized: *C. agastachys*, *C. bequaertii* (including *C. mossii*), *C. microcarpa*, *C. pendula* and *C. penduliformis* that constituted monophyletic groups in a previous

molecular phylogentic reconstruction (Míguez *et al.*, 2017). Interestingly, the phylogenetic relationships between species differ from morphogroups retrieved in this study: *C. microcarpa* and *C. pendula* were retrieved as the most morphologically similar (see Fig. 2-D), despite the fact that they are not sister taxa in the phylogenetic analyses (Míguez *et al.*,in press). It is somewhat unexpected given that *C. agastachys* and *C. pendula*, have traditionally been treated as a single species. Here *C. penduliformis* is yielded as the most morphologically similar to *C. agastachys*. These results indicate either some degree of morphological stasis or convergence. The later are often the result of similar ecological pressures (Martín-Bravo *et al.*, 2007; Givnish, 2010).

Taxonomic consequences

Although *Carex* sect. *Rhynchocystis* is a morphologically quite homogeneous group, our study reveals differences between species that agree with recently shown phylogenetic relationships (Míguez et al. 2017).

The high degree of morphological differentiation between *C. agastachys* and *C. pendula* (Fig. 1-B; Table 3), as well as the ancient split between the two taxa (c. 10 Ma, Miocene; Míguez et al.in press) is enough to justify their consideration as different species. It is striking that despite the clear-cut differences, both taxa had previously remained unnoticed. This might be due to their mostly allopatric distribution with contact in an undetermined area in central Europe, drawing a fake continuous distribution. A nomenclatural study (Jiménez-Mejías et al., 2017) revealed that the type of the name *C. agastachys*, traditionally subsumed within *C. pendula*, matches the diagnostic characters here reported for the species, thus we propose to resurrect this name for this long unnoticed taxon.

Right on the contrary, the geographical distribution misled the conception of *C. bequaertii* and *C. mossii* as different species. Nelmes (1940) described the species *C. mossii as* "scarcely specifically distinct from *C. bequaertii*", and emphasized their distinctiveness mainly relying on their different distributions: *C. bequaertii* inhabited East Tropical Africa, whereas *C. moosii* was regarded as distributed in South Africa (Gordon-Gray, 1995; Gehrke, 2011). It apparently makes sense because such disjunction might limit gene flow via geographical isolation, frequently causing allopatric speciation (Gavrilets, 2003). However, *C. bequaertii* and *C. mossii* were not differentiated in our phylogenetic study (Míguez et al., 2017) and only very subtly in our morphological evaluation (Figs 2-D, 3). Only a qualitative character seems to be clear-cut, the glume coloration. In addition two quantitative characters (ACHW and MAXCLPM; Fig. 4) may help to distinguish between the two taxa, but despite being their mean values significantly different, they show an overlap > 25%. It suggests that divergence between the two sets of populations is very recent and thus morphological differentiation incipient. However the slight differences between the two taxa are somewhat emphasized by their geographical separation (morphogeographic compartimentalization; Stuessy, 1990). According to all this, we propose to consider the two taxa as a single species with two subspecies: *C. bequaertii* ssp. *bequaertii* and *C. bequaertii* spp. *mossii*.

Our study did not find any important incongruence between the phylogenetic and morphological patterns regarding *C. microcarpa* and *C. penduliformis*. Gehrke (2011) indicated morphological resemblance between the Malagasy endemic *C. penduliformis*, *C. bequaertii* and *C. mossii*, but it referred mostly to the morphological homogeneity among *Carex* sect. *Rhynchocystis* species rather than true lack of differentiation (Fig. 2-A; Table 3).

Summarizing, we propose to treat *Carex* sect. *Rhynchocystis* as containing five species: *C. agastachys, C. bequaertii*, *C. microcarpa*, *C. pendula* and *C. penduliformis*, and to consider two subspecies within *C. bequaertii*: subsp .*bequaertii* and subsp. *mossii*. Our analyses revealed clear-cut diagnostic characters neglected by the traditional taxonomy of the group, whereas we found that other characters were overvalued and not diagnostic. This study illustrates that even within a relatively well-studied area as Europe, plant taxonomy may benefit from objective and careful evaluations.

Taxonomic treatment

For the preparation of the morphological synopsis we have relied in our own data but also, in order to be as exhaustive as possible, in the information provided in previously treatments (Kükenthal, 1909; Chermezon, 1923; De Wildeman, 1927; Nelmes, 1940; Maire, 1976; Jermy et al., 2007; Haines & Lye, 1983; Gordon-Gray, 1995; Kukkonen, 1998; Egorova, 1999; Reznicek, 2001; Luceño, 2008; Gehrke, 2011). Same applies to distribution, ecology and phenology. According to the terminology proposed by Jiménez-Mejías et al. (2016) we refer to the prophyll enclosing the flower as a utricle, and to the prophyll embracing the base of the spike as a cladoprophyll. Distributions by botanical countries are summarized according to TDWG codes (Brummit, 2001).

Carex* sect. *Rhynchocystis Dumort., Fl. Belg. 147. 1827.

Typus: *Carex maxima* Scop. (=*C. pendula* Huds.), the section described as monotypic.

Description: Perennial, densely cespitose, with short strong rhizomes, usually forming large ± conspicuous tussocks. Stems 40–230(300) cm long × 2–6.0 mm in diameter,

sharply trigonous, smooth or scabrid distally, sometimes reddish-purple at base. Leaf blades shorter than stem, (8)10–20 mm wide, dark green or yellowish green above, somewhat glaucous beneath; linear, smooth or scabrid on margins or towards the tip, flat to M-shaped in cross section, usually with 2 adaxial lateral veins more prominent that midvein, abaxially only the midvein prominent; ligule present, membranous, reddish or hyaline, becoming darker when dry, apex acute, subacute or emarginate; sheath fronts reddish, greenish or hyaline, veined; basal sheaths weak, entire and scale-like but promptly decomposing in fibers. Inflorescences racemose, with 5–8 spikes, only one per each node, the 1(2) uppermost one(s) erect or slightly arching, entirely male or also with female flowers at top, base or at the middle, the 2–8 lateral ones usually very long, from arching to pendulous, entirely female or very shortly androgynous; proximal bracts leaflike, long-sheathing. Males spikes, when present, terminal, sessile, erect, long and narrowly cylindrical, reaching 35–180 × 4–7 mm. Lateral spikes 60–160(260) mm, terete or narrowly fusiform, flexuous, densely flowered, usually with up to 100 utricles or more, the utricles spreading or slightly ascending, proximal-most spikes usually long-pedunculate, distal-most ones with the peduncle progressively shorter, all with a tubular cladoprophyll at the base. Staminate glumes 3–7 × 0.2–1.5 mm, linear lanceolate or spatulate, brown with green midrib, with or without hyaline margins, apex mucronate or aristate, sometimes sparsely and irregularly erosed. Pistillate glumes 1.9–5.6 × 0.5–2.3 mm, oblong, narrowly obovate or narrowly ovate, mucronate or awned, reddish-brown, sometimes straw-coloured when dry, with a greenish or hialine midrib. Stigmas 3. Utricles 2–4 × 0.8–1.7mm, elliptic or ovate, obtusely and usually asymmetrically trigonous, smooth, glabrous, with 2 strongly marked marginal veins, veinless or weakly veined on faces, cuneate and sessile at base, the apex ± abruptly contracted into a beak up to 0.5 mm, ± cylindrical, smooth, truncate

or shortly bidentate, the teeth less than 0.1 mm. Achenes elliptic, obovate or ovate, trigonous, much smaller than the utricles, ± cuneate at base and often contracted into a substipitate base, rounded at apex, with the style base persistent, shortly cylindrical.

Etymology: from the Greek *rhynchos*, beak, and *kystis*, bladder, probably in reference to the shortly-beaked utricles.

Distribution: Southwestern Palearctic including North Africa and Macaronesia (Azores and Madeira), mountains of East Tropical Africa and eastern South Africa, Madagascar.

Observations: The fact that *C. agastachys* and *C. pendula* have been treated as conspecific to date poses a problem with certain data attributed to *C. pendula*. *Carex agastachys* and *C. pendula* co-occurs through an undefined overlap area through Central and Eastern Europe, west to central Germany, east to Hungary (Attila Mesterházy, pers. comm.), and south to the western Balkan peninsula. *Carex agastachys* lays east from this contact area, whereas *C. pendula* lays mostly west and south of it. In Turkey, the two species seems to become completely allopatric, and we confirmed that the populations of the northern half are *C. agastachys*¸and those of Mediterranean Turkey are *C. pendula*. According to this mostly allopatric distribution, we consider that reports from localities west-south or east the contact area belong respectively to *C. agastachys* and to *C. pendula*, although further confirmation would be desirable. The report of 2*n*=62 for *C. pendula* (Druskovic, 1982) could not be assigned to *C. agastachys* nor *C. pendula* since no information is provided about the provenance of the sample.

In the same sense, the reports of *C. pendula* as an introduced species must be taken with caution. *Carex pendula* in its broad sense has been reported introduced from North America [CAL ORE VRG WAS] and New Zealand [NZS]. Plants introduced in New Zeland are *C. pendula* (K. Ford pers. comm). However, our observations on plants from North America revealed that both, *Carex pendula* and *C. agastachys* are present in the U.S.A. Critical examination of materials would be needed to figure out the identity of the introduced plants in other localities. In addition, some of the studied cultivated materials display a quite unstable combination of the characters of the two species, which might point to a hybrid origin for the plants sold in American nurseries. Additional studied are needed to confirm the hybrid status of these plants.

KEY TO THE SPECIES OF *CAREX* SECT. *RHYNCHOCYSTIS*

1. Uppermost spikes with male and female flowers, very rarely entirely male; body of the pistillate glumes conspicuously longer than the utricles … ***C. bequaertii***

> **1.a.** Pistillate glumes brown, usually with a wide middle central nerve lighter than the scale sides; achenes (1)1.4–1.9(2.1) × (0.3)0.7–0.9 mm; ligule subacute, sometimes emarginated … ***C. bequaertii*** ssp. ***bequaertii***
>
> **1.b.** Pistillate glumes pale brown, with a ± narrow middle nerve usually darker than the scale sides; achenes 1.4–1.8 × 0.8–1mm; ligule emarginated … ***C. bequaertii*** ssp. ***moosii***

1´. Uppermost 1–2 spikes entirely male; body of the pistillate glumes equaling or shorter than the utricles, rarely slightly longer … 2

2. All spikes erect or the lowermost one slightly spreading, subsessile or the lowermost with a peduncle up to 50 mm; leaves strongly coriaceous; ligule from acute to obtuse; stems 40–100 cm tall … ***C. microcarpa***

2´. At least the lowermost spike conspicuously pendulous when mature, with a peduncle (0)25–100(160) mm; leaves herbaceous, soft, non-coriaceous; ligule acute or subacute; stems usually more than (50)100 cm tall … 3

3. Uppermost 3–6 spikes sessile, male and female ones separated by very short internodes, appearing like an aggregate cluster of spikes tipping the inflorescence; mature utricles and achenes dark-brown to blackish … ***C. penduliformis.***

3´. Uppermost spikes pedunculate, with apparent internodes between them, occasionally only the 2 uppermost male spikes (when more than 1) closely approximate; mature utricles and achenes greenish, yellowish, or light-brown … 4

4. Achenes obovate, with the widest point near the top; ligule conspicuously red or reddish-purple; peduncle of the lowermost spike conspicuously scabrid; utricle beak bidentate or truncate … ***C. agastachys***

4´. Achenes oval, with the widest point at the middle or slightly above it; ligule whitish, becoming brownish when dry, rarely slightly reddish-tinged; peduncle of the lowermost spike smooth or very sparsely scabrid; utricle beak truncate … ***C. pendula***

1. *Carex agastachys* L.f., Suppl. Pl. 414 (1782).

Ind. Loc.: "Habitat in Germania" [Germany].

Type: 19 Agastachys. *Carex agastachys* L., Hannoverae, *F. Ehrhart* [Phytophylacium ehrhartianum]. *Lectotype:* LINN-HS1441-174-1!, designated by Jiménez-Mejías et al. (in press); *isotypes* GOET!, M!

=*Carex mutabilis* Willd., Fl. Berol. Prodr. 37 (1787).

Ind. Loc.: "Spandau hinter due Schülerbergen" [Germany].

Type: Willdenow, 1787, Fl. Berol. Prodr., Tab. II, Fig. 6. *Lectotype* designated by Jiménez-Mejías et al. (in press). *Epitype:* B-W-17248! (Willdenow's herbarium at B), designated by Jiménez-Mejías et al. (in press).

Iconography: Fig. 6.

Description: Stems 50–90 cm × 2–7 mm, densely scabrid distally. Leaf blades 9–16 mm wide; ligule 10–27(28) mm, red or reddish-purple, acute; basal sheaths faint and absent, the stem bases covered by old-leaf remnants, dark ferrugineous-red. Inflorescence with 1(2) male spikes at the apex, and (3)4–6 lateral female spikes, exceptionally shortly androgynous, the lower ones often distant; lowermost bract leaf-like, usually larger than the inflorescence. Male spikes 23–110(140) × 3–8 mm, fusiform or cylindrical, erect, spreading or pendulous, sessile or with a peduncle up to 55 mm. Lateral spikes 62–170 × 3–6 mm, long cylindrical, flexuose, spreading or at least the lowermost one pendulous, with peduncles 15–85(100) mm, scabrid. Staminate glumes (3.7)4.3–6.5× 0.4–1.1mm, linear-spatulate, apex ciliate, dark reddish brown. Pistillate glumes (1.9)2–2.6 (2.7)× (0.5)0.6–1.2(1.3)mm, oblong or narrowly elliptic, mucronate, usually shorter than utricle, reddish brown with a light green to white midrib. Utricles 2–3 × 0.5–1.3 mm, narrowly ellipsoid, yellowish green to brownish green when mature, sometimes with sparse small elongated purplish spots, beak conspicuously bidentate or truncate. Achenes 0.7–1.5 × 0.3–1.2 mm, markedly obovate, brownish.

Distribution: Central Europe west to Germany, Balkan Peninsula, Carpathians, Crimea, northern Anatolia, Caucasus, and Alborz range in northern Iran; there are additional problematic records from Afghanistan; introduced at least in North America in

Washington State [AFG? AUT BUL CZE_CZ CZE_SL GEO GER HUN IRN KRY NCS POL ROM TCS_AR TCS_AZ TUR UKR was YUG_SE YUG_SL], see also comments under the section heading.

Habitat: Moist woods and streams; 60–1000 m.

Phenology: March–August.

Etymology: From the Greek *aga*, large, and *stachys*, spike.

Chromosome number: 2*n*=58 (Hindakova, 1978).

Observations: The World Checklist of the Cyperaceae (2017) points to the presence of "*C. pendula*" in Afghanistan. Because of geographic consistency, these materials might be *C. agastachys* and not *C. pendula*, However, we have not been able to trace any material or published record that support that statement. The easternmost populations that we have studied are from mountains on the eastern shores of the Caspian Sea in Iran, which are about 630 km away from the Afghanistan border. Such citation might also be a punctual introduction (perhaps of *C. pendula*) or simply the result of a misidentification. Therefore, the presence of species of section *Rhynchocystis* in Afghanistan should be considered doubtful and needed of revision.

A specimen from Turkey (E-00305402!) is recorded in Nilson (1985) as problematic: "female spikes with several small branches at the base, arising through sterile utricles". After the study of this material, we confirm that the ligule and female spike peduncle characters of these plants match *C. agastachys*, but the undeveloped achenes seem to be

elliptical, as in *C. pendula*. We consider these plants to probably be malformed specimens of *C. agastachys*.

We have confirmed that the reports of introduced *Carex pendula* in Washington State are actually *C. agastachys.*

2. *Carex bequaertii* De Wild., Pl. Bequaert. 4: 246 (1927).

Ind. Loc.: "Ruwenzori mountains, vallée du Lanuri" [Democratic Republic of Congo]

Type: DR Congo, Ruwenzori Mts., Lanuri Valley 3000 m, *J.C.C. Bequaert* 4677. *Holotype:* BR-863827B!; *isotypes:* BR! K!

Iconography: Fig. 7.

Description: Stems 60–200 cm × 2–4 mm, smooth. Leaf blades 7–21 mm wide; ligule 5–25 mm, hyaline, subacute or emarginate; basal sheaths subcoriaceous, keeled, dark brown. Inflorescence with 6–9 pendulous spikes, the uppermost spike mostly with female flowers, with male flowers scattered and diversely mixed between the female ones, very rarely entirely male, the rest of the spikes entirely female or rarely androgynous; lowermost bract leaf-like, equaling or slightly shorter than the inflorescence. Spikes 60–220 × 5–10 mm, cylindrical, pendulous when mature, with peduncles up to 16 cm long, smooth. Staminate glumes (3)4–6 × 0.5–1.2(1.8) mm, lanceolate, apex acute the frequently, shortly awned, brown with a pale midrib. Pistillate glumes 2.6–5.4 × 0.9–1.5 mm, deltoid-lanceolate, oblong-lanceolate or lanceolate-elliptic, acute, subulate, mucronate or awned, longer and narrower than the utricles, brownish with a distinct midrib. Utricles 2.2–3.4 × 0.9–1.5 mm, ovate or elliptic, green yellowish green or blackish, with a short bidentate beak. Achenes (1)1.4–1.9 (2.1) ×

(0.3)0.7–1.1 mm, from obovate to elliptical, yellowish brown to brown, sometimes with dark spots.

Distribution: Mountains of eastern tropical and southeastern Africa.

Habitat: Swamps and wet and moist soils, often along streams and lake shores, also in shady forests; 500-4000 m.

Phenology: January–December.

Etymology: Dedicated to J.C.C. Bequaert (1886–1982), an American naturalist of Belgian origin, who collected the type material.

Observations: The name *C. petitiana* A. Rich have been sometimes used to refer to *C. bequaertii* (e.g. Kükenthal 1909). The confusion was already noted by Gehrke (2011).

2a. *Carex bequaertii* subsp *bequaertii*

=*Carex bequaertii* var. *maxima* Lye, Nordic J. Bot. 3 (2): 244 (1983).

Ind. Loc.: "Uganda, Karamoja district, Mt. Morungole" [Uganda]

Type: Uganda, Karamoja district, Mt. Morungole, 2440 m, iv.1960, *J. Wilson* 1012. *Holotype:* EA; *isotype*: K!.

= *Carex robusta* Hochst. in Schimp, Pl. Abyss. 1: 100 (1850).

Carex robusta Hochst. ex Boeckeler, Flora 40: 411 (1876), pro syn.

Ind. Loc.: not explicit [Ethiopia].

Type: Flora Abyssinica 100 *Carex robusta* Hoschst, in montibus Debra Eski, 11000, *Schimper*, 27 Octobre 1850. *Lectotype* (here designated): P01825501!

Iconography: Fig. 7(A-B, D-I).

Description: Stems 2.5–3 mm wide. Ligule subacute, sometimes emarginated. Pistillate glumes deltoid-lanceolate, acute, the proximal-most ones subulate, brown, with a distinct paler midrib. Achenes 1–2.1× (0.3)0.7–1 mm.

Distribution: Mountains of eastern tropical Africa, North to Simien mountains, West to the Rwenzori, and South to the Kilimanjaro. [CON ETH KEN TAN RWA TAN UGA].

Habitat: Swamps and wet and moist soils, along streams and lake shores. 2200–3700 m.

Phenology: March–November.

Chromosome number: 2*n*=58 (Hedberg & Hedberg, 1977).

2b. *Carex bequaertii* subsp *mossii* (Nelmes) Míguez, Gehrke, Martín-Bravo & Jim.-Mejías, comb. nov.

≡*Carex mossii* Nelmes, Bull. Misc. Inform. Kew 1940: 137 (1940) [basionym]

Ind. Loc.: South Africa, Eastern Cape Province, Hogsback [Republic of South Africa]

Type: South Africa, Eastern Cape Province, Hogsback, 01.i.1927, *C. E. Moss* 999. *Holotype:* K!; *Isotype*: BR.

Iconography: Fig. 7(C).

Description: Stems 2.5–4.0 mm wide. Ligule emarginated. Pistillate glumes oblong-lanceolate or lanceolate-elliptic, pale brown, margin narrowly hyaline, with the midrib usually darker than the sides, apex acute mucronate. Achenes 1.4–1.8 × (0.5)0.8–1.1 mm.

Distribution: Drakensberg mountains in Southeastern Africa [CPP NAT TVL].

Habitat: Wet and moist soils, along streams or in shady forests; 500–2000 m.

Phenology: October–December (February)

Etymology: Dedicated to C. E. Moss (1870–1930), British botanist who collected the type material.

3. *Carex microcarpa* Bertol. ex Moris, Stirp. Sard. Elench. 1: 48 (1827).

Ind. Loc.:"in montibus juxta rivulos" [Sardinia, Italy, not explicit].

Type: Juxta rivulos in montibus Sardinia, Aprili, Majo. *Lectotype*: FI! (specimen at the right), designated by Arrigoni (1984).

=*Carex corsica* Degl. ex Loisel. in J.L.A.Loiseleur-Deslongchamps, Fl. Gall., ed. 2, 2: 307 (1828).

Ind. Loc.: "In Corsica; juxta Bonifacio" [Corsica, France]

Type: Not found, probably at AV (Stafleu & Cowan (1976–1997).

Iconography: Fig. 8.

Description: Stem 40–100 cm × 2–3 mm, smooth or scabrid. Leaf blades 4–9mm wide, strongly coriaceous; ligule 4–30 mm, hyaline, from acute to obtuse; basal sheaths inconspicuous, the stem bases covered by old-leaf remnants. Inflorescence with 1(3) male spikes at the apex, and 4(5) lateral spikes, sometimes 1–2 of them shortly androgynous; lowermost bract leaf-like, shorter or equaling the inflorescence. Male spikes 58–105 × 3–5 mm, fusiform, erect, with a peduncle 10–30 mm. Lateral spikes 75–110 × (3)4–7 mm, female or shortly androgynous, cylindrical, erect or the lowermost one slightly spreading, sessile or subsessile, occasionally the lowermost one with a peduncle up to 50 mm. Staminate glumes 4.8–6.9 × 0.7–1.2 mm, lanceolate, acute or acuminate, brown with a hyaline midrib. Pistillate glumes 2.7–3.7 × 0.9–2 mm, ovate-lanceolate, acute, equaling or slightly longer than the utricles, reddish-brown, with a greenish midrib. Utricles 2–3.3 × 0.8–1.4 mm, elliptic, pale green and purplish-brown puncticulate, beak bifid. Achenes (1.4)1.5–1.7(1.9) × (0.7)0.9–1.1(1.3) mm, obovate, dark-brown.

Distribution: Corsica, Sardinia, Elba Island and central Italian Peninsula [COR ITA SAR]

Habitat: Marshes and wet meadows; 0–1800 m.

Phenology: March–April(August).

Etimology: From the Greek *mikros*, small, and *karpos*, fruit.

Chromosome number: 2n=60 (Contandriopoulos, 1962).

Observations: The lectotype designated by Arrigoni (1984) bears two stems. The left one is *C. pendula*. The right one, which must be considered the lectotype, is *C. microcarpa*.

4. *Carex pendula* Huds., Fl. Angl.: 352 (1762).

Ind. Loc.:"Habitat in sylvis et sepibus humidis; in sepibus inter Hampstead et Highgate copiose" [England, United Kingdom].

Neotype: Morison, 1699, Pl. Hist. Univ. Oxon. 3m sect. 8, tab. 12, fig. 4. designated by Egorova (1999). *Epitype*: London. Hampstead Heath, between Hampstead and Highgate, Ken Wood lake –vc 21, Middlesex, *M. A. Spencer* MAS-2012-040 (BM001074530!), designated by Jiménez-Mejías et al. (in press); *isoepitype*: UPOS!

= *Carex maxima* Scop., Fl. Carniol., ed. 2, 2: 229 (1772)

Ind. Loc.: Not explicit [Carniola, Slovenia].

Type: G. A. Scopoli, s.n. *Lectotype:* LINN-HL110-94!, designated by Jiménez-Mejías et al. (in press).

= *Carex pendula* var. *myosuroides* Boott, Ill. Gen. Carex 4: 197 (1867).

- *Carex myosuroides* Lowe, Trans. Cambridge Philos. Soc. 4(1): 10 (1833), nom. illeg.

Ind. Loc.: "Hab. In Maderae ora septentrionali" [Madeira, Portugal]

Type: Not found, probably at BM (Stafleu & Cowan (1976–1997)).

Iconography: Fig. 9.

Description: Stems 50–180(240) cm × 2–6 mm, smooth or slightly scabrid distally. Leaf blades (6)8–19 mm; ligule 12–65 mm long, whitish, hyaline, becoming brownish when dry, rarely slightly reddish-tinged, acute to subacute; basal sheaths faint, scale-like, dark brown or reddish, often the stem bases covered by old-leaf remnants, pale brown. Inflorescence with 1(2) male spikes at the apex, and (4)6–8 lateral female spikes, exceptionally shortly androgynous; lowermost bract leaf-like, equaling or slightly shorter than the inflorescence. Male spikes 60–160(180) × (2.5)4–9 mm, fusiform or cylindrical, erect, spreading, or pendulous, sessile or subsessile, sometimes with a peduncle up to 2.5 cm. Lateral spikes 85–190(260) × (3)6.5–8 mm, long-cylindrical, flexuose, spreading or pendulous, subsessile or with peduncles 20–100 mm, usually smooth, rarely sparsely scabrid. Staminate glumes 3.6–6.9(8.7) × 0.2–1.9 mm, linear, oblong or narrowly obovate, acute, reddish-brown with a hyaline midrib. Pistillate glumes (2.2)2.4–3.7(3.9) × 0.6–1.2 mm, narrowly ovate to narrowly obovate, mucronate, the body generally shorter than the utricles or shortly surpassing them, reddish-brown with a greenish midrib. Utricles (1.4)1.9–3.6 × 0.5–1.5 mm, ovoid or ellipsoid, greenish or yellowish green, beak 0.2–0.5 mm, truncate. Achenes (1)1.1–1.8(2.1) × (0.4)1.4–1.5 mm, elliptical, with the maximum width at the middle or slightly above it, brownish to yellowish.

Distribution: Europe and the Mediterranean, including northwestern Africa and the Mediterranean shores of southwestern Asia, in Europe north to Denmark and east to

central Germany, and western Hungary, also in the Atlantic archipelagos of Azores and Madeira; apparently introduced in southern Scandinavia, and confirmed also as introduced in the southern island of New Zealand, and also in North America at least in Oregon (see comments under the section heading); a problematic record from Iraq [ALG AZO BGM COR CYP DEN EAI FRA GER GRB GRC BEL DEN HUN IRE IRQ? ITA KRI LBS MDR MOR NET ore nzs PAL? POR SAR SIC SPA swe SWI TUN TUR YUG_CR YUG_MN YUG_SL].

Habitat: Riparian forests, beside streams, usually on damp clayish soils. 25–1370 m.

Phenology: (March)April–August(December).

Etymology: From the Latin *pendulus,* hanging, in reference to the pendulous lateral spikes.

Chromosome number: 2n=58(Kjellqvist & Löve, 1963; Löve & Kjellqvist, 1973; Strid & Franzen, 1981), 60(Ottonello et al., 1985).

Observations: We have not seen materials from the East Aegean Islands, Ireland and Palestine, but because of geographical consistency we consider that these materials very probably belong to *C. pendula* and not to *C. agastachys* (see comments at the beginning of the treatment).

We have examined the voucher cited in Flora of Iraq (Hooper, 1985) (see Appendix 1) and its identity points to *C. pendula* (ligule whitish, female spike peduncles smooth). However, the identification is not entirely conclusive, since it is an immature specimen.

The Iraqi locality of *C. pendula* is highly isolated, as the closest *Carex* sect. *Rhynchocystis* populations seem to be about 350 km northeast in the Iranian Alborz Mountains near the Caspian Sea (*C. agastachys*), about 500 km northwest in the Turkish Pontic Mountains between the Black Sea and the Caucasus (*C. agastachys*), and about 700 km west in the Levant coast (*C. pendula*). We cannot rule out an one-time introduction of *C. pendula* in the area, although it would certainly be unexpected. For further comments on introduced plants the reader is referred to the comments under the section heading.

5. *Carex penduliformis* Cherm. Bull. Soc. Bot. France 70: 414 (1923).

Ind. Loc.: "Forêt d'Andasibé […], Massif de l'Andringitra" [Madagascar]

Type: Madagascar Centre, forêt d'Andasibé (basin de l'Onive), novembre 1911, *H. P. de la Bâthie* 2535. *Lectotype* P!, designated by Gehrke (2011). *Isolectotypes*: P!. *Paratype:* Madagascar, Centre, Andringitra Mts., 1922, *H. P. de la Bâthie* 14554, P!

Iconography: Fig. 10.

Description: Stem 60–100 cm × 2–3 mm, smooth to scabrid. Leaf blades 9–12 mm wide; ligule 17–30 mm, reddish, subacute; basal sheaths scale-like, dark brown or ferruginous-red. Inflorescence with 1 male spike and 6–7 sessile female or shortly androgynous spikes aggregated at the apex, and 2–3 distant lateral long-peduncled female or shortly androgynous spikes; lowermost bract leaf-like, usually exceeding the length of the inflorescence. Male spikes 120–138 × 3.3–4.5 mm, fusiform, erect, spreading or pendulous, sessile or subsessile. Female and androgynous spikes 160–230 × 4.5–5mm, long cylindrical, flexuose, spreading or at least the lowermost ones

pendulous and with a peduncle 2–4 cm, scabrid, Staminate glumes 5.1–6.4 × 1.1–1.5mm, ovate to lanceolate, aristate or mucronate, brown with a lighter midrib. Pistillate glumes 1.9–3.6 × 1–1.3 mm long, ovate, mucronate or aristate, the body shorter than the utricles, dark brown, with a lighter midrib and no hyaline margin. Utricles 2–2.4 × 0.8–0.9mm, elliptic, dark-brown, sometimes red-punctate, beak truncate or very shallowly bidentate. Achenes 1–1.5 × 0.8–0.9mm, obovoid to elliptical, dark-brown.

Distribution: so far only known from North and East Madagascar [MDG].

Habitat: Wetlands and stram edges (1350–2600m)

Phenology: Plants in flower and fruit are only known from November.

Etymology: Resembling *C. pendula*.

Observations: The doubtful reports of *Carex boryana* Schkuhr from Madagascar (see Escudero & Luceño, 2011) seems to correspond to *C. penduliformis*, as some of the studied specimens bear labels with the name "*Carex boryana*", as originally identified by de la Bâthie.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supporting Information S1. Scatter plot of principal components extracted from the PCA as described in Figure 1. All the species of section *Rhynchocystis* (PCA-I): PC1 vs PC3(A); *C. agastachys* and *C. penduliformis* (PCA III): PC-1 vs PC-3(B) and PC-1 vs PC-4(C); *C. microcarpa* and *C. pendula* (PCA IV)*:* PC-1 vs PC-3(D); *C. bequaertii* and *C. mossii* (PCA V): PC-1 vs PC-3(E) and PC-1 vs PC-4(F). Symbols depict the different taxa considered: *C. agastachys* =black circle, *C. bequaertii* =black triangle, *C. microcarpa* = black square, *C. mossii*=white triangle, *C. pendula*=white square and *C. penduliformis*=white circle.

Supporting Information S2. Main results of the Discriminant Function Anlysis (DFA) resulting from, *Carex* sect. *Rhynchocystis* (DFA-I) and *C. bequertii-C. mossii* (DFA-II), datasets studied.

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Figure Legends

Figure 1. Schematic representation of the datasets and analyses performed in our hierarchical approach to study morphological variation in *Carex* sect. *Rhynchocystis*. Preliminary Data Exploration (PCA-I) was performed using 13 variables and the 109 specimens to explore the complete dataset (*C. agastachys, C. bequaertii, C. microcarpa, C. mossii, C. pendula and C. pendulifomis*). Subset 1 (PCA-II) was perfomed using six variables and 69 specimens to explore dataset containing *C. agastachys, C. microcarpa, C. pendula* and *C. penduliformis*. Subset 1.A (PCA-III) was performed using 13 variables and 28 specimens to explore data set containing *C. agastachys* and *C. penduliformis*. Subset 1.B (PCA–IV) was performed using13 variables and 43 specimens to explore dataset containing *C. microcarpa* and *C. pendula*. Subset 2 (PCA-V) was performed using 13 variables and 39 specimens to explore dataset containing *C. bequaertii* and *C. mossii* subset 2.

Figure 2. Scatter plot of the first two principal components extracted from the PCA as described in Figure 1. A (PCA-I)- All the species of section *Rhychocystis*; B (PCAII)- C. *agastachys, C. microcarpa, C. pendula* and *C. penduliformis*; C (PCA III)- *C. agastachys* and *C. penduliformis*; D (PCA IV)- *C. microcarpa* and *C. pendula*; E (PCA V)- *C. bequaertii* and *C. mossii*. Symbols depict the different taxa considered: *C. agastachys* =black circle, *C. bequaertii* =black triangle, *C. microcarpa* = black square, *C. mossii*=white triangle, *C. pendula*=white square and *C. penduliformis*=white circle.

Figure 3. Boxplots of the most discriminant characters retrieved by DFA or with less than 25% overlap. The X-axis represents the considered species labelled as follow: AGA (*C. agastachys*), BEA (*C. bequaertii*), MIC (*C. microcarpa*), MOS (*C. mossii*),

PEN (*C. pendula*), PFO (*C. penduliformis*). The boxes cover 50% of the data values ranging between the 25th and 75th percentiles, and the lines show 90% of the values between the fifth and 95th percentiles. The line within the box represents the median. Outlying values are indicated by small "o's" and far out values are indicated by asterisks (*)

Figure 4. Histograms of the qualitative variables: Distal ligule color (0 whitish, 1 pale brownish, 2 reddish-purple); Proximal ligule color (0 whitish, 1 pale brownish, 2 reddish-purple); Relative position on the lowermost spike (0 if the spike is pendulous, 1 if it is erect); Sex of upper spike (0 if an upper completely male spike is absent, 1 if it is present) as scored for the morphometric study of the six considered species. Species are abbreviates as follow: AGA (*C. agastachys*), BEA (*C. bequaertii*), MIC (*C. microcarpa*), MOS (*C. mossii*), PEN (*C. pendula*), PFO (*C. penduliformis*). X-axis represents the measurement and Y-axis the frequency.

Figure 5. Scanning electron micrographs of the entire achene (A, C, E, G, I, K; scale bar 100 µl) and detail of the achene surface (B, D, F, H, J, L; scale bar 10 µl) in *Carex* section *Rhynchocystis*. *C. agastachys* (A, B); *C. bequaertii* (C, D); *C. microcarpa* (E, F)*; C. mossii* (G, H); *C. pendula* (I, J); *C. penduliformis* (K, L).

Figure 6. Analytical illustration of *Carex agastachys* L.f. Serbia, S Carpatians, Djerdap National Park. 21 June 2010. P. Jiménez-Mejías 86PJM10. UPOS-4208. (A) Culm base; (B) leaf apex; (C) ligule; (D) inflorescence; (E) proximal spike peduncle ; (F) male

spike; (G) staminate glume; (H) female spike; (I) pistillate glume; (J) utricle; (K) achene. Drawing by F. Míguez.

Figure 7. Analytical illustration of *Carex bequaertii* DeWild. ssp. *bequaertii* (A-F, H-I) Kenya: Aberdares National Park. 26 July 2007. M.L: Buide et al. 82UPO-K, UPOS-3288; and ssp. *mossii* (G) South Africa, KwaZulu-Natal, Ntabamhlophe. 10 November 2012, E. Maguilla et al. 50EMS12(BIS5), UPOS-5195. (A) Culm base; (B) ligule; (C) inflorescence; (D) lateral spike; (E) staminate glume; (F) pistillate glume of subsp. *bequaertii*; (G) pistillate glume of subsp. *mossii*; (H) utricle; (I) achene. Drawing by F. Míguez.

Figure 8. Analytical illustration of *Carex microcarpa* Moris. (A-B, D-I) France, Corsica, Ghiosimi. 4 May 2007, M. Escudero 88ME07, UPOS-4720. (C) France, Corsica, Ghiosimi 5 May 2007. M. Escudero 104ME07, UPOS 4723. (A) habit; (B) ligule (C) inflorescence; (D) male spike; (E) staminate glume; (F) female spike; (G) pistillate glume; (H) utricle; (I) achene. Drawing by F. Míguez.

Figure 9. Analytical llustration of *Carex pendula* Huds. (A, B, D, I, J) Italy, Tuscany. 13 June 2010. P. Jiménez-Mejías 24PJM10, UPOS-4136; (F, H) France, Cévennes national Park. 25 June 2009. P. Jiménez-Mejías 104PJM09, UPOS-5878; (C, E, G) Italy, Piedmont, 8 June 2012. P. Jiménez-Mejías & E. Martinetto 64PJM12, UPOS-5348. (A) culm base; (B) leaf apex; (C) ligule; (D) inflorescence; (E) male spike; (F)

staminate glume; (G) female spike; (H) pistillate glume; (I) utricle; (J) achene. Drawing by F. Míguez.

Figure 10. Analytical illustration of *Carex penduliformis* Cherm. Madagascar, Mahajanga, Bealanana, Mangindrano, Ambohimirahavavy, Bemafo, Campement 02 Bemafo. S. Wohlhauser et al. 795, P-01874870. (A) Culm base; (B) ligule; (C) inflorescence; (D) male spike; (E) staminate glume; (F) female spike; (G) pistillate glume; (H) utricle; (I) achene. Drawing by F. Míguez.

Table 1: Comparison of the main treatments of *Carex* section *Rhynchocystis* Dumort and the treatment derived from our own results.

This study	**Global *Carex* Group (2016) Section *Rhynchocystis* Dumort.**	**Egorova (1999) Section *Rhynchocystis* Dumort.**	**Kükenthal (1909) (=Sect. *Maximae* Asch.)**	**Natural distribution**
C. agastachys L.f	(included in *C. pendula*)	(included in *C. pendula*)	(included in *C. pendula*)	Central Europe to the Caucasus and northwestern Iran
C. bequaertii DeWild subsp. *bequaertii*	*C. bequaertii* DeWild	*C. bequaertii* DeWild *C. petitiana* A. Rich[1]	*C. petitiana* A. Rich[1]	Eastern Tropical Africa, north to Ethiopia and south to Tanzania
C. bequaertii subsp. *mossii*	*C. mossii* Nelmes	*C. mossii* Nelmes	*C. mossii* Nelmes	Eastern parts of Southern Africa

(Nelmes) Míguez et al.				
C. microcarpa Moris	*C. microcarpa* Moris	*C. microcarpa* Moris	*C. microcarpa* Moris	Corsica, Sardinia and C Italian Peninsula
C. pendula Huds.	*C. pendula* Huds.	*C. pendula* Huds.[2]	*C. pendula* Huds.	Central and western Europe, Mediterranean Basin, and Macaronesia
C. penduliformis Cherm.		*C. penduliformis* Cherm.		Madagascar
			C. joorii L.H. Bailey[3]	Southeast U.S.A. to Texas
			C. shortiana Dewey[3]	South Ontario to North-central and Eastern U.S.A.
			C. jaluensis	Russian Far East to Korea

	Kamarov[4]	
	C. maculata Boott[4]	Tropical and Subtropical Asia to South-West Pacific
	C. vicinalis Boott[4]	South India

[1]*C. petitiana* was used to refer to *C. bequaertii* as Gehrke (2010) already noted. [2]According to our results, *C. pendula* is absent from the area covered by Egorova's (1999) treatment, thus all the natural populations this work refers to are actually *C. agastachys*. [3]The North American *C. joorii* and *C. shortiana* were transferred to sections *Glaucescentes* and *Shortianae*, respectively (Reznicek, 2001, which is supported by recent phylogenies (Carex Global Group, 2016). [4]The Asian *C. jaluensis* and *C. maculata* have been transferred to section *Anomalae* (Dai et al., 2010); accordingly, the character displayed by *C. vicinalis*, endemic from S India (e.g. bracts sheathless), places this species as a member of section *Anomalae* too, as already recognized in Global *Carex* Group (2016).

Table 2. Variables included in the analyses performed and scores obtained in the first plotted principal components of the PCA analyses of the different subsets of *Carex* sect. *Rhynchocystis*. Species are abbreviated as follows: aga (*C. agastachys*), bea (*C. bequaertii*), mic (*C. microcarpa*), mos (*C. mossii*), pen (*C. pendula*), pfo (*C. penduliformis).*

		PCA-I Preliminary Data Exploration (aga, bea, mic, mos, pen, pfor)			**PCA-II** Subset 1 (aga, mic, pen, pfor)		**PCA-III** Subset1.A (aga, pfor)				**PCA-IV** Subset 1.B (mic, pen,)			**PCA-V** Subset 2 (beq, mos)			
		PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC1**	**PC2**	**PC3**	**PCA 4**	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**	**PC4**
variable	**Continuous variables**																
ACHL	Achene length	0.573	0.547	0.247	**-0.768**	0.257	**0.818**	0.297	0.142	-0.2688	0.619	0.243	-0.4652	0.192	**0.773**	0.080	0.036

ACHW	Achene width				**0.807**	0.316	0.179	-0.088	0.507	0.442	0.179	-0.009	**0.750**	0.306	-0.197
ACL	Length from the achene base to the maximum width zone	0.453	0.460	0.486	**0.811**	-0.055	0.165	0.086				0.322	**0.789**	0.096	-0.103
CLMW	Culm width											-0.246	-0.353	-0.328	-0.214
DSFS	Distance between superior female spikes	**-0.729**	0.257	-0.058											
FSLP	Peduncle length of the proximal female spike														

INFLF	Female length	Spike									-0.644	-0.013	-0.572
INFLM	Male length	Spike											
INFWF	Female width	Spike	0.695	-0.121	0.303		0.568	0.490	0.323	-0.090			
INFWM	Male Spike width												
LIGL	Ligule length		-0.524	0.118	**0.559**								
LUMWD	Length from the utricle base to the maximum width zone		0.599	0.512	-0.104	-0.586	**0.703**				**0.754**	0.402	0.062

MAXCLPM	Maximum length of the pistillate scale colored margin				-0.223	**0.780**	-0.261	-0.071	-0.569	0.538	-0.079				
PSCLL	Pistillate scale length	**0.822**	-0.054	-0.073								0.661	-0.270	0.167	0.426
PSCLW	Pistillate scale maximum width														
SLEAFW	longlest leaf width available on the sheet				-0.521	0.207	0.514	-0.454							
SSCLL	Staminate scale length														
SSCLW	Staminate scale maximum width				0.408	**-0.730**	-0.170	-0.140							

								9	6	5							
UBL	Utricle beak length						0.527	-0.4099	-0.3895	0.165							
UL	Utricle length	0.609	0.534	0.108	**-0.730**	0.555	0.547	0.465	-0.4595	-0.125	0.660	0.605	-0.237	0.377	0.096	-0.259	**0.814**
UMW	Utricle maximum width						0.435	0.686	-0.0311	0.178	0.463	0.685	0.224	0.217	0.430	-0.495	-0.042
UPLDS	Utricle peak length from the distal part of the spike										-0.647	0.313	0.467	0.495	-0.146	0.585	-0.007
UPLMS	Utricle peak length from the										**-0.73**	0.111	0.326	0.588	-0.38	0.464	-0.31

	middle part of the spike									**5**				7		9
UPLPS	Utricle peak length from the proximal part of the spike									-0.509	0.417	0.127	0.690	-0.269	0.359	-0.101
USL	Utricle stalk length	0.690	0.132	-0.372		0.325	-0.624	-0.082	0.049	0.699	-0.105	-0.016	-0.449	0.073	0.529	0.469
	Discrete variables															
PSDFS	Peduncle scabrousness of the distal female spike				0.591	0.290	-0.288	0.654	-0.070	0.614						

PSPFS	Peduncle scabrousness of the proximal female spike												
SPKFN	Female/Androginous Spike number	0.687	-0.3444	0.012		0.284	-0.3335	0.630	0.504				
SPKMN	Male Spike number	**-0.761**	0.405	0.061									
	Qualitative variables												
DLC	Distal Ligule color (0 whitish, 1 pale			**0.776**	0.526					0.796	-0.105	-0.335	-0.179

	brownish, 2 reddish-purple)											
PLC	Proximal Ligule color (0 whitish, 1 pale brownish, 2 reddish-purple)			**0.720**	0.604	**0.753**	-0.242	0.272	0.765	0.001	-0.422	0.008
RPLS	Relative position on the lowermost spike (0 if the spike is pendulous, 1 if it is erect)	-0.050	**0.598**	-0.574		**0.716**	-0.482	0.283				
SUS	Sex of upper spike (0 if an upper	**0.888**	-0.331	-0.086								

	completely male spike is absent, 1 if it is present)

Table 3. Characters that showed less than 25% overlap in pairwise comparisons at species level marked by an asterisk (abbreviations specified in Table 1). Characters found to be significantly different by the Mann-Whitney U test are marked with a hash. Qualitative characters marked with a plus sign.

	C. agastachys	*C. bequaertii*	*C. microcarpa*	*C. mossii*	*C. pendula*
C. bequaertii	ACHL#, ACL#*, DLC+, DSFS#, DSFS#, FSLP#*, INFLM#, INFWM#, LIGL#*, LUMWD#*, MAXCLPM#*, PLC+, PSCLL#*, PSCLW#*,				

	PSDFS#, PSPFS#, SLEAFW*, SPKFN#, SPKMN#, SSCLL#, SUS+, UBL#, UL#, UPLDS#	
C. microcarpa	ACHL#*, DLC+, MAXCLPM*, PLC+, PSCLL#*, PSCLW#*, PSDFS#, PSPFS#, SLEAFW#*, UPLPS#	ACHW*, DSFS#, FSLP#*, INFLF*, INFLM#, INFWM#, PSCLL#*, SLEAFW#*, SPKFN#, SPKMN#, RPLS+, SSCLL#*, SSCLW#, SUS+, UPLPS#

C. mossii	ACHL#*, ACL#*, DLC+, DSFS#, INFLF#*, INFLM#, INFWM#, LIGL#*, LUMWD#*, MAXCLPM#, PLC+, PSCLL#*, PSCLW*, SDFS#, PSPFS#, SPKFN#, SPKMN#, SUS+, UL#, UPLDS#	ACHW#*, MAXCLPM#	ACHL#, DSFS#, FSLP#, INFLF#, INFLM#, INFWM#, PSPFS#, SLEAFW#*, SPKFN#, SPKMN#, RPLS+, SSCLL#*	
C. pendula	ACHL#*, DLC+, DSFS#, INFLF#*, INFLM#*, INFWM#, PSCLL#*, LIGL#*, PLC+, PSCLW#, PSDFS#, SPKFN#	ACL*, DSFS#, FSLP#, INFLM#, INFLM#, INFWM#, LIGL#*, LUMWD#, MAXCLPM#, PSCLL#*, PSCLW#*,	INFLF#, INFLM#, LIGL#*, MAXCLPM#, PSCLL#, PSPFS#, SLEAFW#*, RPLS+, UPLDS#,	ACL*, DSFS#, INFLM#, INFWM#, LIGL#*, PSCLL#, PSCLW*,

		PSDFS#, PSPFS#, SPKFN#, SPKMN#, SSCLL#*, SUS+, UPLDS#	UPLMS#, UPLPS#	PSDFS#, PSPFS#, SPKFN#, SPKMN#, SSCLL#, SCLW#, SUS+, UPLDS#	
C. penduliformis	ACL*, DSFS#, FSLP*, INFLF#*,INFLM*, LIGL*, MAXCLPM*, PSCLW*, SLEAF*, UBL#	ACHW*, DLC+, FSLP*, INFLF*, INFLM#, INFWM#, LIGL*, LUMWD*, PLC+, PSCLL*, PSPFS#, SLEAFW*,	ACHL*, DLC+, FSLP*, INFLM*, LIGL*, PLC+, PSPFS#, RPLS+, SLEAFW*	ACHL*, DLC+, INFLM#, INFWM#, LIGL*, MAXCLPM*, PLC+,	ACHL*, ACL*, DLC+, DSFS#, INFLM*, MAXCLPM#*, PLC+, SLEAFW*

	SPKMN#, SUS+,	SSCLL*,	PSPFS#, SPKMN#, SLEAFW*, SUS+, SSCLL*, UL#

Appendix . Representative Specimens Examined of *Carex* sect. *Rhynchocystis*. The 109 numbered sheets are those used in the morphometric study. The rest of the sheets indicated with an asterisk were examined carefully although they were not introduced in the morphometric examination.

Carex agastachys — **ARMENIA**: *SE of Kapan, 16 Jun 2016, Więcław& Jac. Koopman, 89 (Herbarium Jac. Koopman 3506). — **AUSTRIA**: **1** Attersee area, village Unterach, SW of Stockwinkel, 28 May 1996, E.Vitek 96-244 (M-0177712). **2** Village Unterach am Attersee, SW of Stockwinkel, 28 May 1996, E. Vitek 96-244 (UPS-V149677).*Village Unterach am Attersee, SW of Stockwinkel, 28 May 1996, E. Vitek 96-244 (BM-1122551) **3** Vorarlberg, 25 Jun 74, W. Lippert 15024 (M-0177708). **4** Unterinntal, 17 Jun 1976, A. Polatschek s.n. (P-01735578). **5** Tirol, Wallchsee-Kranzach, 26 Aug 1968, W. Lippert 166563. (M-0177709). *Stiria media, May 1910, H. Sabransky (RBGE).*Kapfenstein, Fehring, 6 Jun 1966, J. Höller (M-0177710).*Fürstenbrunn, 17 Jul 1996, F. Eberlein MTB 8243/4.2 (M-0177711).— **AZERBAIJAN**: **6** Mesazhai, 7 May 91, N.V. Kostylëva & N.V.Kostyleva, s.n. (MHA). **7** Lekoransky Zone. Alekseyevka, 27 May 58, V.G.Eropob, s.n. (MHA).* Tuapse, 28 Aug1976, P. A. Kapmconoba s.n. (MHA). — **BULGARIA**: **8** Burgas, M. StrandZa, N. Vihodcevsky, s.n. (UPS-V571923). — **CZECH REPUBLIC**: **9** Moravia Centralis, 10 Jun 62, J. Dvorák, s.n. (M-0151978). **10** Zlínský, Vlára, 29 Jul 1932, J.PodpÊra 994 (UPS-V571922). *Zlínský, Moravia orient., 29 Jun 1928, G. Říčan (UPS-V571921).— **GERMANY**: **11** Oberbayern Kreis, Traunstein; Ruhpolding, 22 Jun 1903, (M-0177732). **12** Bayern, Kreis Unterllgäu, 1 Jul 1984, W.Lippert 20008. (M-0177736). **13** Kreis traunstein, 12 Jun 1988, W.Lippert 23598. (M-0177733). **14** Baden-Württemberg,

Heidelberg, 02 Jun 84, Fr. Hörl, s.n. (M-0177726). **15** Bavaria, Kreis Freising; St. Alban, 11 Aug 1980, J.Sellma MTB 7837/3 (M-0177729).*Lausitzer Bergland, 6 Jul 1914, Weder 431 (M-0177714). *Lindau, im Rickenbacher Tobel, 22 May 1904, G. Hoock (M-0177735). *Bavaria, Rothenbuch, Ohmüller.(M-0177731). * Staffelsee sw, 8 Jul 1961, J.Höller (M-0177738). *Beuerberg nw, 29 Jun 1965 (M-0177737).*Oberweier in Baden, 13 May 1899, A. Kneucker (M-0177713).*Markinshabel, 13 Jun 1884, Briecke 6942 (M-0177705).*Überhängende, 9 Aug 1923, Beck (M-0177734).— **IRAN**: **16** Golestan, Bender Ges: prope Wonischtepe, 24 Mar 1901, P. Sintenis 1455 (UPS-V571920). **17** Gilan, Navrud (Asalem) to Herowabad, 15 May 71, J. Lamond 2967 (E-00305405).*Gilan, 17 May 1971, K.H.Rechinger 39760 (M-0151970). *Mazanderan, 11 Jun 1966, J.C Archibald 2292 (E-00305403).*Navrud (Asalem), 15 May 1971, J. Lamond (2967). — **POLAND**: *Beskid Sadecki, 20 June 2009, A. Tyc, EG3503 (KRA-427512). — **SLOVAKIA**: **18** Australis, distr. Banska Stiavnica, Jun 1898, S.Kupcok, s.n. (P-02231326).*Pozsony-Szentgyörgy, 36 May 1912, F. Filarszky & S. Jávorka (M-0151975).— **SERBIA**: **19** Bor District, Djerdap National Park. Miroc Mountains, 21 Jun 2010, P. Jiménez-Mejías 86PJM10 (UPOS-4208). — **SLOVAKIA**: **20** Pukanec, Bakabánya, 1901, S. Kupeok 27308 (M-0177702). — **SLOVENIA**: **21** LjubljanaPekel-Schlucht, südlich Borovnica, 28 May 66, K.P. Buttler 9566 (M-0177703). **22** Podravksa, Ptju, 09 May 1972, M. Thulin 1974 (UPS-V 571925). — **SWITZERLAND**: *Schweiz kanton Obwalden, 23 May 1940, Wallimann (M-0177706). — **TURKEY**: **23** Trabzon, Trabzon-Macka, 1 Jul 1974, Ansin, R 1476 (E-00449228).*C2 Trabzon, Köprübaşı, 25 Jul 1991, A. Güner et al. 9646 (HUB-33878).*Prov. Bursa: Tahtaköprü, 2 July 1962, Davis & Coode s. n. (E-00305399). *Prov. Lazistan; Distr.Rize, 14 May 1960, Furse & Synge 144 (E-00449222).* A8 Rize de Cayeli: Cürükbel, 5 July 1979, M. Dogan 361 (E-00449226).*Prov. Zonguldak:

Çimsir dere, above Yenice, 20 July 1962, Davis el al. 37832 (E-00449227) — **UKRAINE**: **24** Veliky Berezny, 12-jul-68, A.K.Skvortsov s.n. (M-0151973).*18 jul 1898, A. Kneucker 178 (RBGE).

Carex bequaertii — **CONGO**: **25** Karisimbi volcan, Apr 29, H. Humbert 8053 (P-01825491). **26** Volcan Mikeno, 12 Apr 1905, H. Humbert s.n. (B-100240016).*Kivu, Parc des Virunga, 22 Jan 1972, P.Van der Veken 9124 (Z-00081213). — **ETHIOPIA**: **27** Gara Mullata mountains, 50 km W of Harar, 9 Mar 1969, J.J.F.E De Wilde 4774(P-01825506). *Bale Mountains National Park. Gaysay Valley. B. Gehrke et al., BG241 (Z-000081204). *Oromia, Asella, 7 May 1965, W.J.J.O de Wilde & B.E.E.de Wilde-Duyfjes 6598 (P-01825505). *Bale Mountains National Park, Gaysay Valley.B. Gehrke, BG240 (Z). *South-face of Gara Mullata Mountains, about 50 km W of Harar, 9 Mar 1969, J.J.F.E.De Wilde 4774 (P-01825506). — **KENYA**: **28** Mt Kenya, along bank of Mithi River, 11 Jan 1958, O. Hedberg: from material collected by Coe & Karika 392 (UPS-V571753). **29** Mt Kenya, along river bank of Nithi river, 11 Jan 58, Coe and Karika 392 (UPS-V571743). **30** Mt Elgon, E of koitoboss. 20 Jan 1900, Å. Strid 3553 (UPS-V571758). **31** Mt Kenya, Rift Valley, Nanyuki distr, 16 Jul 71, O. Hedberg 5054b (UPS-V 078660). **32** Rift Valley, N. Cherangani Hills, 3 Aug 1968, M.Thulin & Å. Tidigs 49 (UPS-V571756). **33** Elgon, above Endebess, 10 Jun 1966, R. Wheeler Haines 4165 (P-01825492). **34** Mount Elgon National Park, Koroborte - Koitoboss track (Park Route), B. Gerkhe & M.Muasya BG-145(Z-000081203). **35** Mount Kenya National Park, Sirimon Route path between Old Moses Camp and Shiptons Camp, B. Gerkhe BG-AF98 (Z-000081202). **36** Mount Kenya National Park. Naro Moru Route to Mackinders Camp just at the beginning of the track, 4 Oct 2004, B. Gerkhe, BG-AF79 (Z-000081201). **37** Aberdares National Park, Mutubio, 26 Jul 07, M.L.Buide et al., 82UPO-K (UPOS-3288-1).*Aberdares National Park. Oldonyio Lesatima. M.Muasya &

B.Gehrke, BG-Af 014 (Z-000081207). *Mount Kenya National Park. Naro Moru route, M.Muasya & B.Gehrke, BG79-AF (Z-000081200/01). *Timberless gap, Mt Kenya, 29 Jul 1949, E.A.C.L.E Schelpe 2439 (E-0021940). *Mt Kenya, N. slope, 31 Jul 1975, G. Windels GW107 (Z-000081212).*Nakuru distr, Eastern Mau Forest Reserve, 25 Aug 1949, R.A. Maas 5891 (Z-000081211). *Mount Kenya, NW slopes, 21 Aug 1948, O. Hedberg 2015 (UPS-V571780, UPS-V571781). *Mount Kenya, along bank of Mitchi River, 7 Oct 1961, Coe & Karika 392 (UPS-V571734).*Aberdare Range, Kinangop, 19 Jul 1948, O. Hedberg 1667 (UPS-V571779). *Mount Kenya occidentalis, 4 Feb 1922, Fries & Fries 1388 (UPS-V197078). — **TANZANIA**: **38** Kilimanjaro, 2 Mar 34, H.J.Schlieben 4878 (Z-000081210). **39** Kilimajaro, 2 Mar 34, H.J. Schlieben 4878 (B-100240014).*Masai Distr., Ngorongoro Conserv, 10 Oct 1977, M.D. Leakey 19541(P-01825494).*Bezirk Bagamayo, 20 Jul 1933, J. Schlieben 4188 (Z-000081208).*Kilimajaro; Ngori-walo, 26 Sep 1933, Dr. Gelienger 2712 (Z-0000081215). — **UGANDA**: **40** Mt. Muhavura, 9 Oct 48, O. Hedberg 2261(4/4) (UPS-V571764). **41** Mt. Muahavura, 9 Oct 48, O. Hedberg 2261(1/4) (UPS-V571761). **42** Ruwenzori, Bujuku Valley, 31 Mar 48, O.Hedberg 616(1/2) (UPS-V571766). **43** Ruwenzori, Bujuku Valley, 31 Mar 48, O.Hedberg 616(2/2) (UPS-V571766). **44** western province, Kigezi District, Virunga–Ostgruppe, 8 Nov 54, H.U. Stauffer 716 (Z-000081209). **45** Rift valley Province, Eastern Man Forest Reserve, 25 Aug 1949, R. A. Maas 5891 (PRE-5891). *Bugsihu Distr., Mt Elgon, 4 Dec 1967, O. Hedberg 4558 (UPS-V571760).*Rwenzori Mountains, between Nyabitaba Hut and John Matte Hut. B. Gehrke & H.P. Linder BG352 (Z-000081205). *Rwenzori Mountains, between Nyabitaba Hut and John Matte Hut. 24 Jan 2006, B. Gehrke & H.P. Linder BG352 (Z-000081206). *Rwenzori, Bujuku Valley, 31 Mar 1948, O. Hedberg 616 (UPS-V571768).

Carex microcarpa*— FRANCE**: *Toulon, Apr 1839 (P-01778110), J. Gay. **46** Corse du sud, 7 Jun1885, E. Reverchon 159 (UPS-V571782). **47** Corse du sud, Bonifacio, Jordan s.n. (UPS-V571784). **48** Haute-corse, Bastia ,4 Jun 1869, O. Debeaux s.n. (UPS-V571783). **49** Asco, 6 May 2007, M. Escudero 104ME07-2 (UPOS-4723). **50** Haute-corse, forêt de Vizzavona, 19 Aug 1930, G. Desplantes s.n. (UPS-V571864). **51** Corse du sud, 1 Aug 1930, M. Weiller s.n. (UPS-V571865). **52** Corsica, Calvi, foret de Bonifato, 24 Jun 1961, J. Tatigne s.n. (P-2229528). **53** Haute-corse, Vizzavona, 13 May 1926, M.F. Spencer (UPS-V571785).*Corsica, Ghisome, 4 May 2007, M. Escudero & M. Luceño, 88ME07 (UPOS-4730).*Corsica, Muracciole, 4 May 2007, M. Escudero & M. Luceño, 83ME07 (UPOS-4726).**Corsica, 7 Jun-3 Aug 1885, Reverchon 459 (E-00305392/1).*Corsica, Serra di Scopamene, per Sartène, 3 Jul 1879, E. reverchon159 (RBGE). *Corsica, 20 May 1925, J.Chevalier 4095 (P-01702712).*Corsica, Valleé de la Restorica, 31 Jul 1976, M. Bouby (P01731817). *Corsica, 29 May 1925, J.Chevalier 461 (P-2231671).*Corsica, Serra di Scopamène, 5 July 1879, E. Reverchon (P-02037211, P02038808).*Corsica, Serra di Scopamène, 3 July 1879, E. Reverchon (P-01788097, P-01788098, P-01788099, P-01788100, P-01788101).*Corsica, Forêt d'Aïtone, 7 Jun-3Aug, E. Reverchon (P-01778102, P-01778103, P-01778104).*Corsica, 19 May 1868, O. Debeaux (P-01778105). *Corsica, 1868, Soleirol 25(P-01778106).*Corsica, Bastia, 19 Jun 1949, L.Kralik 825 (P-01778107, P-01778108).*Corsica, Forêt d'Aïtone, 6 Aug 1930 (P02036629).*Corsica, Ghizioni, 4 Oct 1972 (P-02036668).* Corsica, Sources du Pigno, 25 May-7Jun 1866, P. Mabille (P-02037213). P-02038809).*Corsica, Bastelica, 10 Jul 1878 (P-02038807).*Corsica, 1 Jun 1925, D. Chevalier (P-02038810). *Corsica, Vizzavona, 1 Aug 1932, B. de Retz 2908 (P-01974528).*Corsica, Vizzavona, 10 Jul 1930. G. Desplantes (P-01778110).

*Corsica, L´Inzecca, 10 Jul 1930, G. Desplantes (P-01778111).*Corsica, sources du Pigno, 25 May-7 Jun. P. Mabille (P-01778112, P-01778113, P-01778114, P-01778115).*Corsica, Bonifacio (P-01778117).*Corsica, O. Debeaux (P-01778117). *Corsica, 4 Jun 1869, O. Debeaux (P-01778118).*Corsica, Foret de Bonifatto, 23 Jun 1961 (P-01728008).*Corsica, Ghisoni, 27 Jun 1900, R. Rotges 404 (P-02036628).*Corsica, 1 Jul 1882, A. Chevalier 4259 (P-01788095). * Corsica, Monte Cinto, A. Comte et al 625 (PI-4). * Corsica, Foret d´Aitone, 12 Jul 1966, E. K. Horwood (BM-001122548).*— **ITALY**:*Tuscany, Fossetto umido di scolo dei campi (Asciano, Pisa), 1 Apr 1999, M. L Pedulla M.L. (PI-1). **54** Sardinia, Olbia-Tempio, 16 May 1882, E.Reverchon 230 (UPS-V571867).*Sardinia, Ogliastra. Rio Pira, 12 Jun 2013, Urbani & Calvia C1 (SS).*Sardinia, Ogliastra. Baumela river, 12 Jun 2013, Urbani & Calvia D1 (SS). I. Müller s.n. (E-00305390).*Sardinia, Arrondissement de Tempio, 16 May, Reverchon (P-01666311, P-01997847).*Sardinia, Monte Limbardo, 2 Feb 1833, Reverchon (P-01839322, P-01839325).*Sardinia, E. Cosson (P01858289).* Sardinia, 16 May 1883, M.L. Barbara (P01997848).*Sardinia (P01839323, P01839324).*Sardinia 10 Jun 1882, E. Reverchon 3300 (BM-001122547).

Carex mossii — **SOUTH AFRICA**: **55** Kwazulu Natal, Parque Natural Mark´s cowl, 14 Dic 2008, M. Luceño 73ML08 (UPOS-4725). **56** Hogsback, Auckland forest reserve, 2 Mar 1987, C. Reid 1204 (UPOS-3080). **57** British Kaffraria, T. Cooper 288 (Z-000080354). **58** Eastern Cape Province, P. MacOwan 1608 (Z-000080355). **59** Hogsback, Auckland forest reserve, 02 Mar 1987, C. Reid 1204 (Z-000081216). **60** Mpumalanga, Barberton; 1933, F.A. Rogers 30212 (Z-0000980356). **61** Kwazulu Natal; Ntabamhlope, 10 Nov 2012, Maguilla et al., 50EMS12bis (UPOS-5195). **62** Traansvaal; Pieteresburg. Woodbush mts, 25 Sep 1927, C.E. Moss 15577 (PRE-0109491). **63** Keiskamma, Eastern Cape, 11 Nov 1948, R. Story 3687 (PRE-01094999). **64**

Kingwilliams rown. Muden Dam, 02 Nov 1942, J.P.H. Acocks 9282 (PRE-0109498). **65** Ohrigstad Dam Nature Reserve, 09 Nov 1971, N. Jacobson 1790 (PRE-0109484). **66** Mount Sheba, 15 Dic 1981, JP, Kluge 2474 (PRE-665646). **67** Ida Doyer Nature Reserve, 27 Jan 1971,A. Loxton 525 (PRE-0109492). **68** Giants Castle Game Reserve, Forest Walk, 04 Feb 85, C. Reid 983 (PRE-762269). **69** Weza Forest reserve, T.K. Lowrey 60417 (PRE-762302). **70** Transkei, 12 Oct 1969, Strey 9179 (PRE-0109500). **71** Paulpietersburg district, Pongola Bush Nature reserve C. Reid 1991 (PRE-762613).*Kwazulu-Natal, Ntabamhlophe, 10 Nov 2012, E. Maguilla et al., 50EMS12 (UPOS-5041).*Transvaal, Ohrigstad Dam Nature Reserve, 9 Nov 1971, N.Jacobson, Jacobson1790 (PRE-019484). *British kaffraria, 1860, T. Cooper 288 (E-00305386, Z-000081219). *British kaffraria, 1862, T. Cooper 3336 (E-00305387). *North of Mthata, 30 Jan 1983, O.M. hilliard & B. L. Burtt 16320 (E-00305385). *Kwazulu Natal. Lions River District, 1991, C. Reid 153 (Z-0000081217). *Easter Cape, Komgha, 1891, H.G.Flanagan, (Z-000081218).

***Carex pendula* — ALGERIA**: **72** Kabylie de Collo, bords des ruisselets, dans la basse vallée de l'Oued Tamanart, près du littoral, 26 May 1944, L. Faurel s.n. (P-01998578). *Argel, 19 Mar1941, L. Faurel. s.n. (P-01998577). *Près d'Alger, 19 Mar 1941, L.Faurel s.n. (P-01998576). — **BELGIUM**: **73** Liège, Seraing, 19 Jun 1999, J.Lambinon 99/248 (M-0177701). * Between Heyd and Villers, 21 Jun 1964, JDeh H74341 (M-0177696). * Braine –le-Comte, 8 May 1869, A. Cogniaux 16 (M-0177698). — **CROATIA**: **74** Licko-senjska: Dalmatien, PlitviCke, 28 Jun 1938, H. Lenander s.n. (UPS-V571926). — **CYPRUS**: * environs de Stavros-tis-Psokas, 21-IV-1991, G. Alziar 0977 (SEV-251911). *environs de Stavros-tis-Psokas, 21-IV-1991, Iter Mediterraneum 977(MA-496245). *Ayia valley, 25 Apr 1962, R.D. Meikle. —**DENMARK**: **75** Bornholm d.47 Vertermarie, Almindingen, 15 Jun 1977, P. Lütken s.n. (P-01889138).

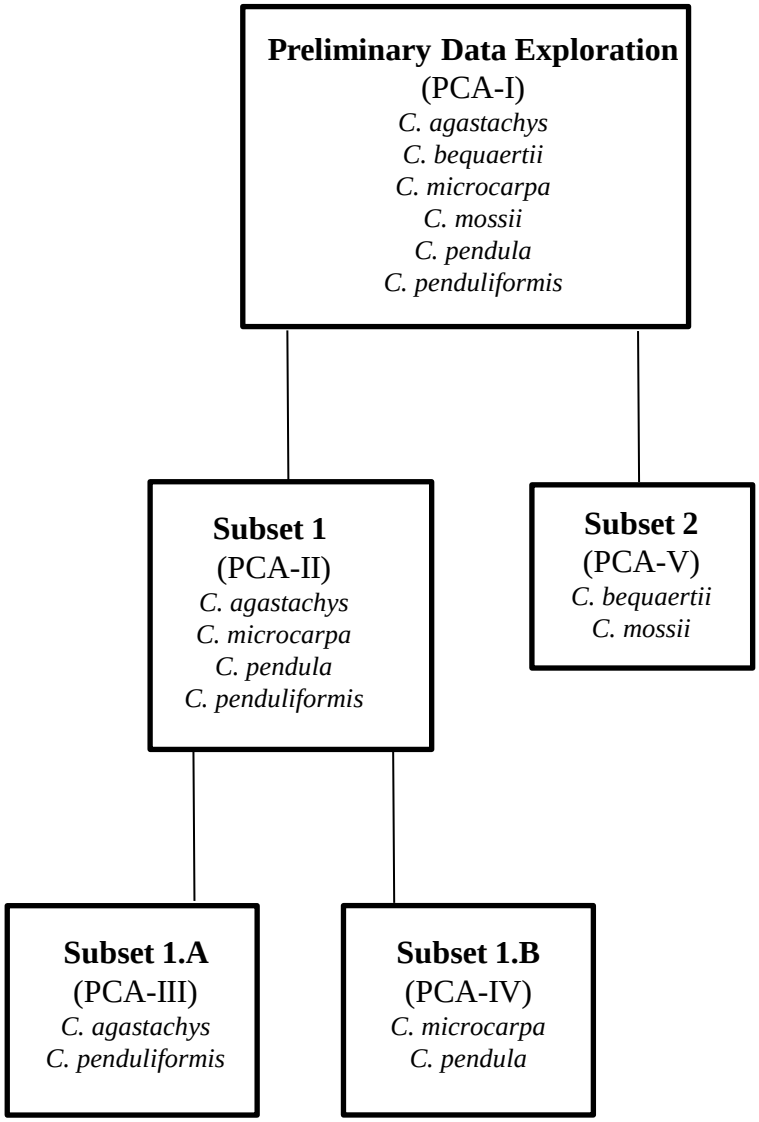
76 Sydjylland, Distr. 52, 22 Jun 1949, I. Segelberg (V-181569). *Sønderjylland : Kollund Skov, 12 Jul 1943, H. S. Dahl (BM-001122550).— **FRANCE**: **77** Cevennes, Road between Les plantiers and Valleraugue, 25 Jun 2009, P. Jiménez_Mejías 104PJM09 (UPOS-5878). **78** Haute Normandie, 8 May 2010, P. Jiménez & J.M. Toro 15PJM10 (UPOS-4099). *Dax, 28 Mar 2006, P. Jiménez-Mejías & M. Escudero 17PJM06 (UPOS-4739). *Forêt de Compiègne, 25 May 1976, G. Sag 530 (P-00044589). *Forêt de Boulogne, 16 Jun 1990, Jac Koopman s.n. (Herbarium J.Koopman).*Languedoc- rosellón, Lozère, 9 Jun 2016, S. Martín-Bravo 36SMB16 (UPOS-8750). — **GEORGIA**: *Georgia, Keda Region, some 30 km east of Batumi, 18 Jun 1999, M. Merello et al 2299 (E-00281678). *Georgia, Abhaeskaya ASSR. Adzharis-Tskhali-env, 20 Jun 1976, E.E.Gogina 1521 (MHA). *Georgia, Kvareli Dagodehsky zapoednik. Gorge Lagodehis-tskapi, 18 Jun 1971, E.E. Gogina 2112 (MHA). — GERMANY: **79** Bayern, Thalkirchen, Golfplatz, 5 Jul 1991, H. Förther 4868 (M-0177739). **80** Baden-Württemberg, Heidelberg, K.P. Buttler 11244 (M-0177720). **81** Saarbrücken, 13 Jun 1910, Ruppert s.n. (M-0177723).*Bord de la rivière Ammer près Ammerthal, 28 May 1979, W. Lippert 16834 (P-01707382). *Ringen, Raeinz 63 (M-0177716). *Bavière, Kreis Weilheim-Schongau, 28 May 1979, W. Lippert & D. Podlech 16834 (M-0177728). * Rügen, 21 Aug 1881, H. Ross (M-0177719). *Schlesurg, Auscker, Hansen (M-017718). * Flensburg, 1876, Prahl (M-0177722).*Mittelrhein, Godesberg, 5-8 Aung 1931, H. Andres 660 (M-0177721). *Südschwarzwald, 2 Jun 1984, Fr.Hörl (M-0177725). *Bergishe Gebiet, 1 Jul 1928, A. Schumacher (M-0177715). *Zabern, May 1885, H. Petry (M-0177717). — **GREAT BRITAIN**: **82** Ile de Jersey, 11 Jun 1939, F. Louis-Arsène s.n. (P-01889136). **83** E. Sussex, Fairlight Glen, near Hastings, C. Bailey 1459 (M-0177699).*Devon, Slapton Ley Field Centre, S. Watson-Jones s.n. (MTMG) *Hertfordshire, The Moors.

Braughing, 8 May 2012, James, TL385255 (Personal herbarium). *London, Hampsted, Heath, Lake Ken Wood, 5 Aug 2012, M.A. Spencer, MAS/2012/040 (UPOS-5004).*Pencalenick, 1937, A.Meebold — **GREECE**: **84** Attica: pr. Baphi, 10 May 1931, F.G. Guiol 1869 (UPS-V571924).*Epiro, Koroni, 11 Jul 2004, P.Vargas & M. Luceño, 261PV04 (UPOS-815). *Trikala, 6 Jul 2007, A.Quintanar et al. AQ 2223 (MA-765137). *Ahaia, 23 Jun 2007, C. Navarro et al. CN 6883 (MA-765823). * Crete, Jania, Langos, 17 May 2005, S. Martín-Bravo & M. Luceño, 378SMB05 (UPOS-242). — **HUNGARY**: **85** a Csikos to Mellett, 12 May 1971, G. Sag 530 (P-02208912). — **ITALY**: **86** Tuscany, alpi apuane, Vinca, 13 Jun 2010, P. Jiménez-Mejías 24PJM10 (UPOS-4136). **87** Elba, Marciana Marina, 9 May 1986 Laubwald s.n. (M-0177704). **88** Piamonte, Turin, Front 8 Jun 2012, P.Jiménez-Mejias & E. Martinetto 54PJM12 (UPOS-5348). *Sardinia, Nuoro, 5 Jun 2003, M. Angel García et al. 3750 (MA-709103). *Sicily, Palermo, 1 Jun 2000, A. Herrero et al. AH 852 (MA-646672). *Messina, 2 Jun 1979 Davis& Sutton 6423 (RBGE). * Torino, Puente de Valle Ceppi, June 2012, P.Jiménez-Mejías et al., 105bisPJM12 (UPOS). — **LEBANON**: *Araya, 26 March 1933, R. Gombault 2998 (P-01899491). *Hamma, 27 Apr 1968, H.C. Stutz 34689 (NY). Araya, 26 March 1933, R. Gombault 2998 (P-P01899491). — **LUXEMBOURG**: **89** Sauergebiet, 25 May 1926, W. Freiberg 1459 (M-0177697). — **MONTENEGRO**: Tivat, 16 Jul 1976, G. Halliday 232/76 (E-00305396). — **MOROCCO**: *Chefchaouèn, 3 Jun 2005, M. Ait Lafkih et al., 61 (BM-340).*Tanger, 25 Jun 1913, C.J. Pitard 1402 (KG-361). — **NETHERLANDS**: *Ressen (GLD), 19 Jun 2010, Jac Koopman s.n. (Herbarium J.Koopman). — **PORTUGAL**: **90** Alentejo, Mora, May 14, R. Palhinha & F. Mendez P6 (LISU-7404). **91** Rib d' Ametado, 14 Aug 1849, Unknow 639 (K-000363418).*Cousá-Quinta de Alfocheria, 29-VIII-1940, R. Palhminha, s.n. (LISU-P7402). *Sintra, J. C. Zamora, s.n. (MA). * Algarve, F. Sales &

I. Hedge 95/36 (E00033730). **92** Azores, Cava do Cabo de Canada, 12 Sep 2013, Shaefer2013/89 (TUM). **93** Azores, Santa Maria, Sao Lorenço,10 Sep 2013, Shaefer2013/90 (TUM).*Azores, Isla de Flores, lagoa dos Patos, 9 Aug 2012, C. aedo 19462 (MA-863075). *Azores, Faial, Caldeira, 29 Jun 1968, E. Sjögren 625 (MA522503). **94** Madeira, Levad Tower in Vamara, 21 Jul 1987, Nobuga 273 (MADJ-05494). **95** North side of Madeira by rivulets, 31 Aug 1837, Boott, J 653 (K-000363419). **96** North Madeira by rivulets, 1837, Boott, J s.n. (K000363417). * Madeira, Santana, Dic 2013, M. Sequeira, MS7806 B (UPOS-5182). — SPAIN: **97** Asturias, Colunga. Playa de la Griega, 5 Aug 2012, F.J. Fernández 10FJF12 2/2 (UPOS-4973). **98** Cáceres, Cascadas Nogaleras, 11 May 2012, M. Maguilla 4EMS12BIS (UPOS-5143).**99** Cáceres, Navalconcejo, 7 May 2011, P. Jiménez-Mejías 52PJM11 (UPOS-4719). **100** Cádiz, Tarifa, Sierra del Bujeo, 23 May 2004, P. Jiménez-Mejias et al. 100PJM04 (UPOS-2163). **101** Huelva, Cueva de la Mora, VI-2004, J.M. Marín 904JMM 1/2 (UPOS-953). **102** Huelva, Cueva de la Mora, VI-2004, J.M. Marín 904JMM 2/2 (UPOS-953). **103** Jaén, Despeñaperros, Aldeaquemada, 24 May 2009, P. Jiménez-Mejías 62PJM09 (UPOS-4720). **104** Sevilla, Cazalla de la Sierra; Parque Natural Sierra Norte Sevilla. 27 Sep 2003, P. Jiménez-Mejías 61PJM03 (UPOS-5877).— **SWITZERLAND**: **105** Lugano, Mendrisio Sw a.D Bahn, 6 Jun 1963, J. Höller s.n. (M-0177707). **106** Ginebra, Terre de Prégnu, pré de fauche, 24 May 2001, R. Piñeiro_Portela s.n. (UPOS-2649). —**TUNISIA**:* Aïn Soltane, 30 May 1950, G. Pottier-Alapetite (UM-MPU-MPU024822). — **TURKEY**: * C2 Muğla, Köycegiz, 20 Apr 1991, A. Güner et al. 8900 et al. (HUB-33881). C2 Muğla, Köycegiz, 20 Apr 1991, A. Güner et al. 9646 et al. (HUB-33880).*Izmir, KemalpaVa, 6 June 1966, R. Alava, 5953 (E-00305406).

Carex penduliformis —**MADAGASCAR**: **107** Antsiranana,Sava, Andapa, Doany, 19 Nov 2005, C. Rakotovao 2518 (P-01874871). **108** Mahajanga,Bealanana, Mangindrano, ambohimirahavavy, Bemafo, Campement 02 Bemafo, S. Wohlhauser et al. 795 (P-01874870). **109** Antsiranana Andapa, Doany, 23 Nov 2005 C. Randrianarivelo & Torize 391 (P-01874872). *****Toliara, 10 Nov 2004, N.M. Andrianjafy et al 637 (P-01999739).*Massif de l'Andringitra, Feb 1922, H.Perrier de la Bâthie 14554 (2/22) (P-00346089). *Massif de l'Andringitra, Feb 1922, H.Perrier de la Bâthie 14554(3/22) (P-00346090). *Forêt d'Andasibé (Bassin de l'Onive), Nov 1911, H. Perrier de la Bâthie 2535 (P-00346091). *Forêt d'Andasibé (Bassin de l'Onive), Nov 1911, H.Perrier de la Bâthie 2535 (P-00346092).

Figure 1


Preliminary Data Exploration
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C. agastachys
C. bequaertii
C. microcarpa
C. mossii
C. pendula
C. penduliformis
Subset 1
(PCA-II)
C. agastachys
C. microcarpa
C. pendula
C. penduliformis
Subset 2
(PCA-V)
C. bequaertii
C. mossii
Subset 1.A
(PCA-III)
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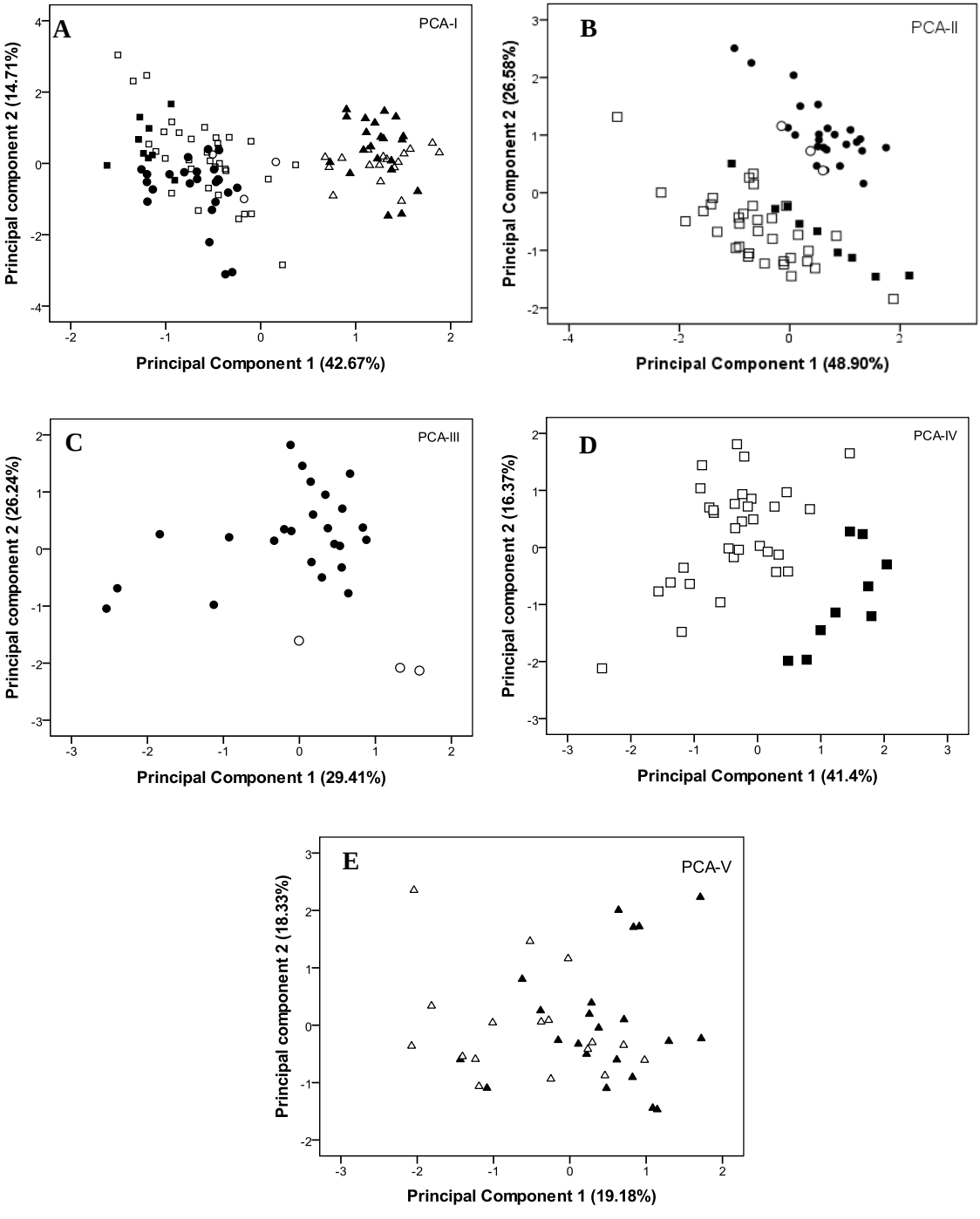
C. penduliformis
Subset 1.B
(PCA-IV)
C. microcarpa
C. pendula

Figure 2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Principal component 2 (14.71%)
Principal Component 1 (42.67%)
B
PCA-II
Principal Component 2 (26.58%)
Principal Component 1 (48.90%)
C
PCA-III
Principal component 2 (26.24%)
Principal Component 1 (29.41%)
D
PCA-IV
Principal component 2 (16.37%)
Principal Component 1 (41.4%)
E
PCA-V
Principal component 2 (18.33%)
Principal Component 1 (19.18%)

Figure 3

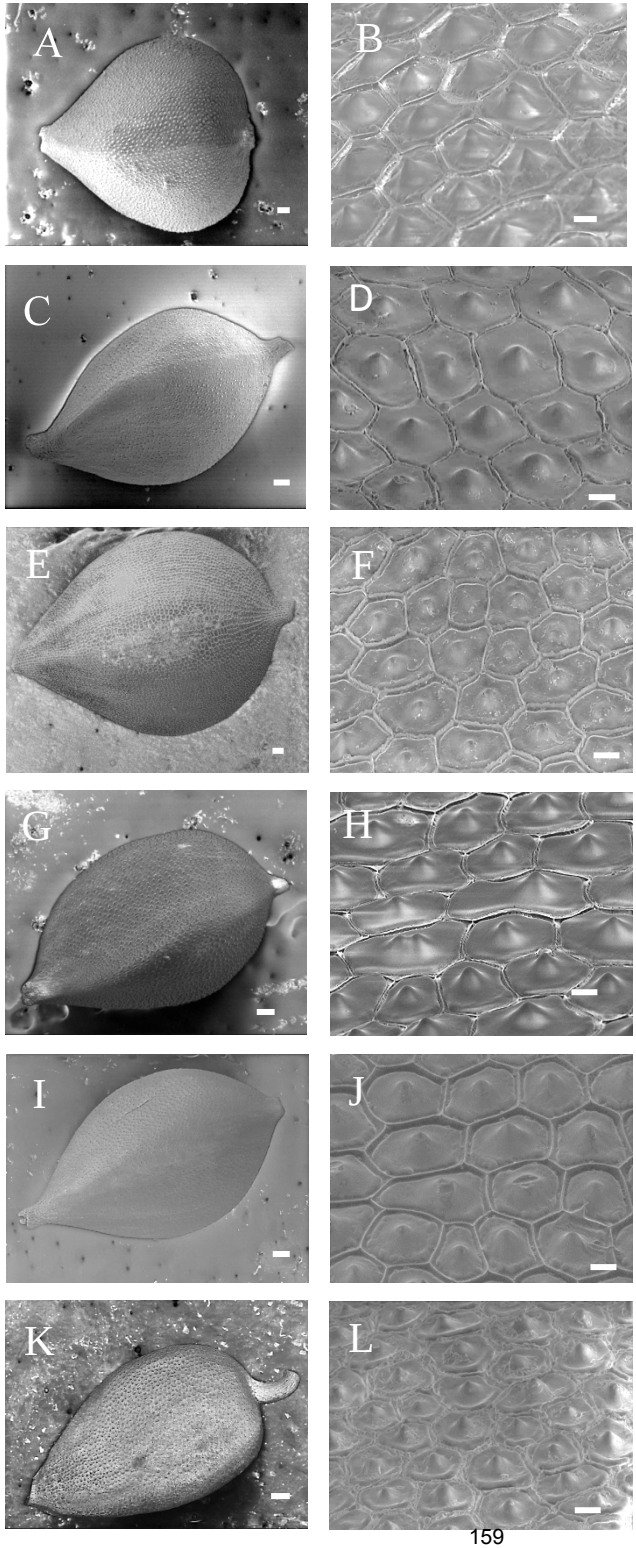
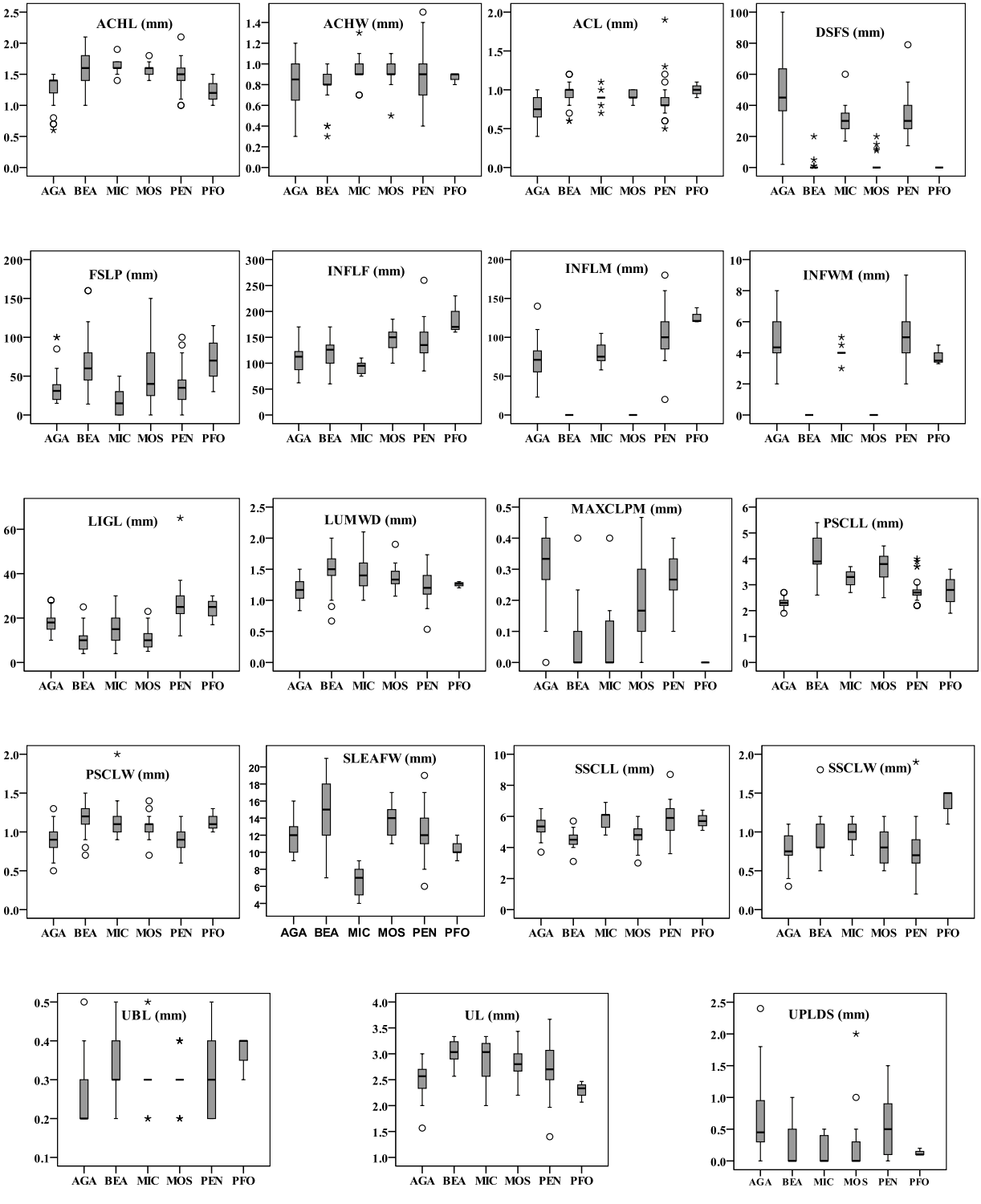


Figure 4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LUMWD (mm)
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AGA BEA MIC MOS PEN PFO

Figure 5

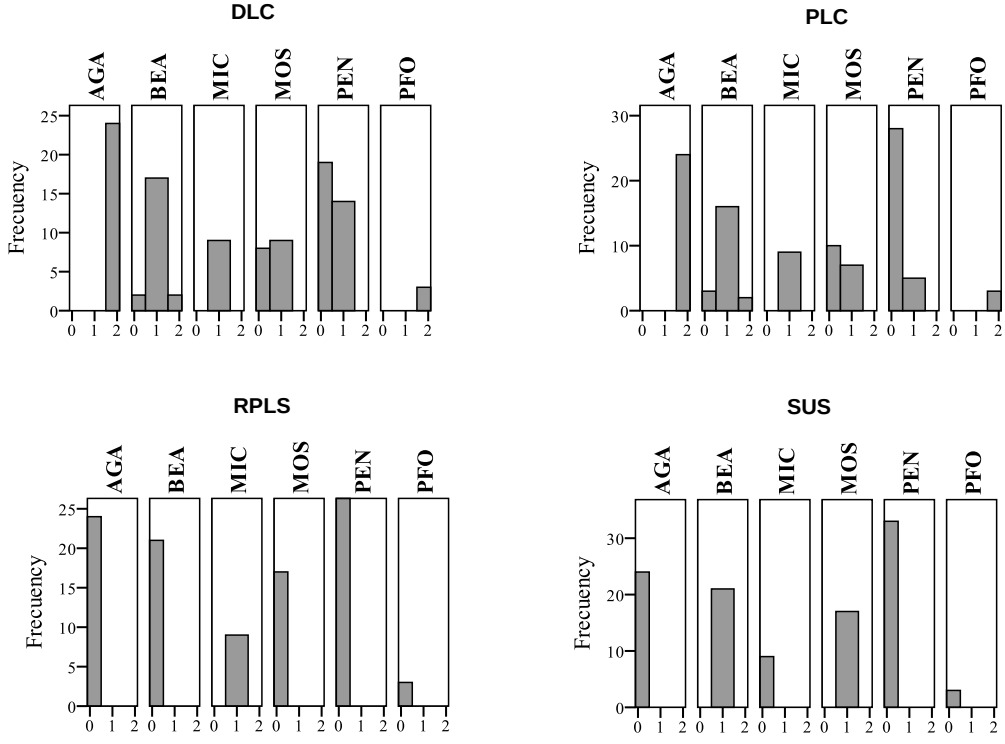
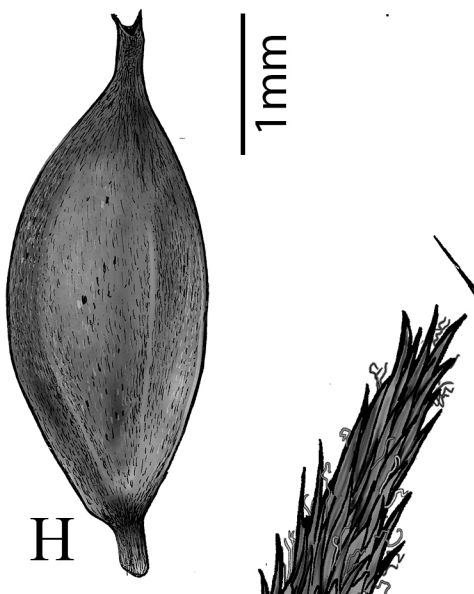
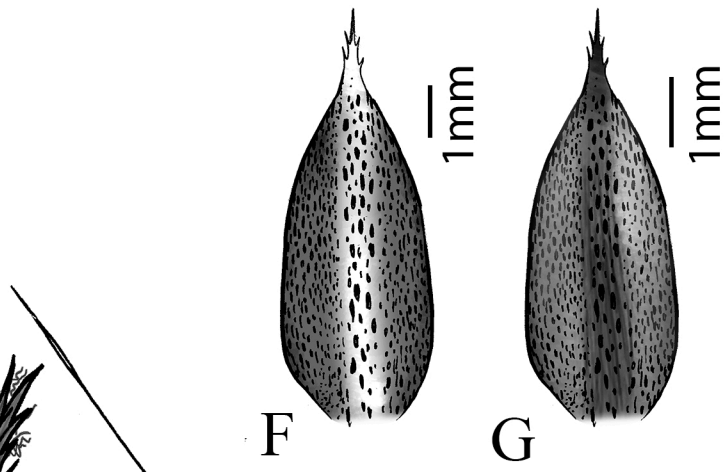
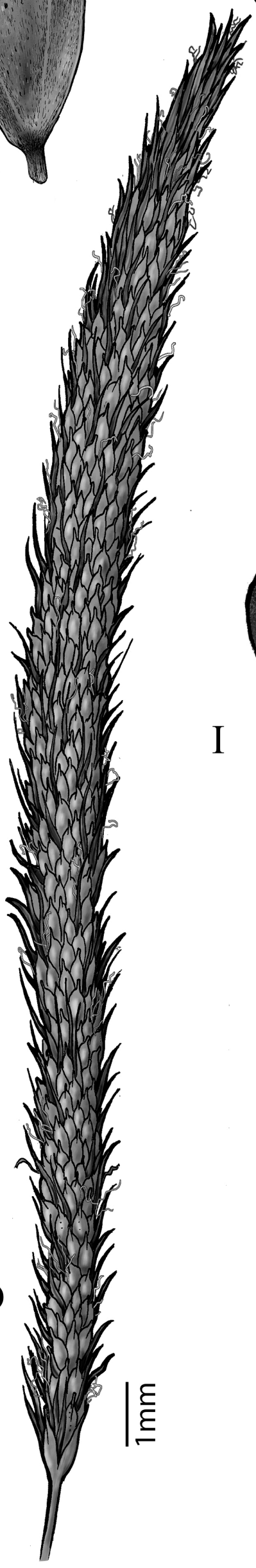


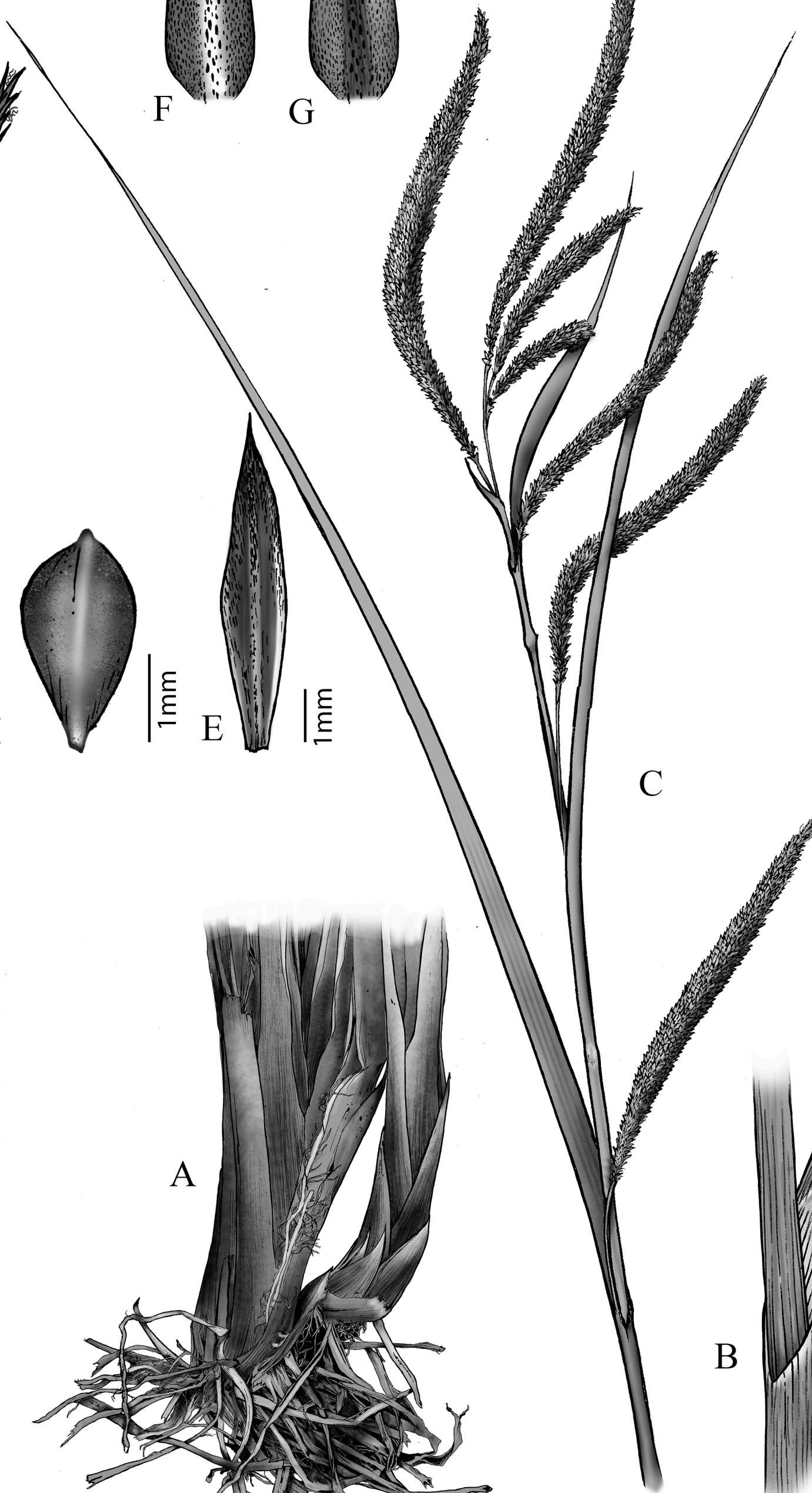
Figure 6

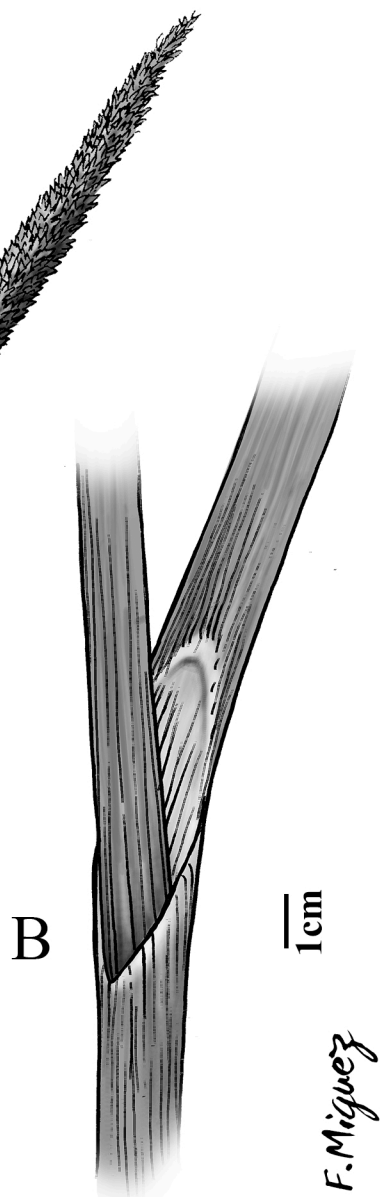
Figure 7











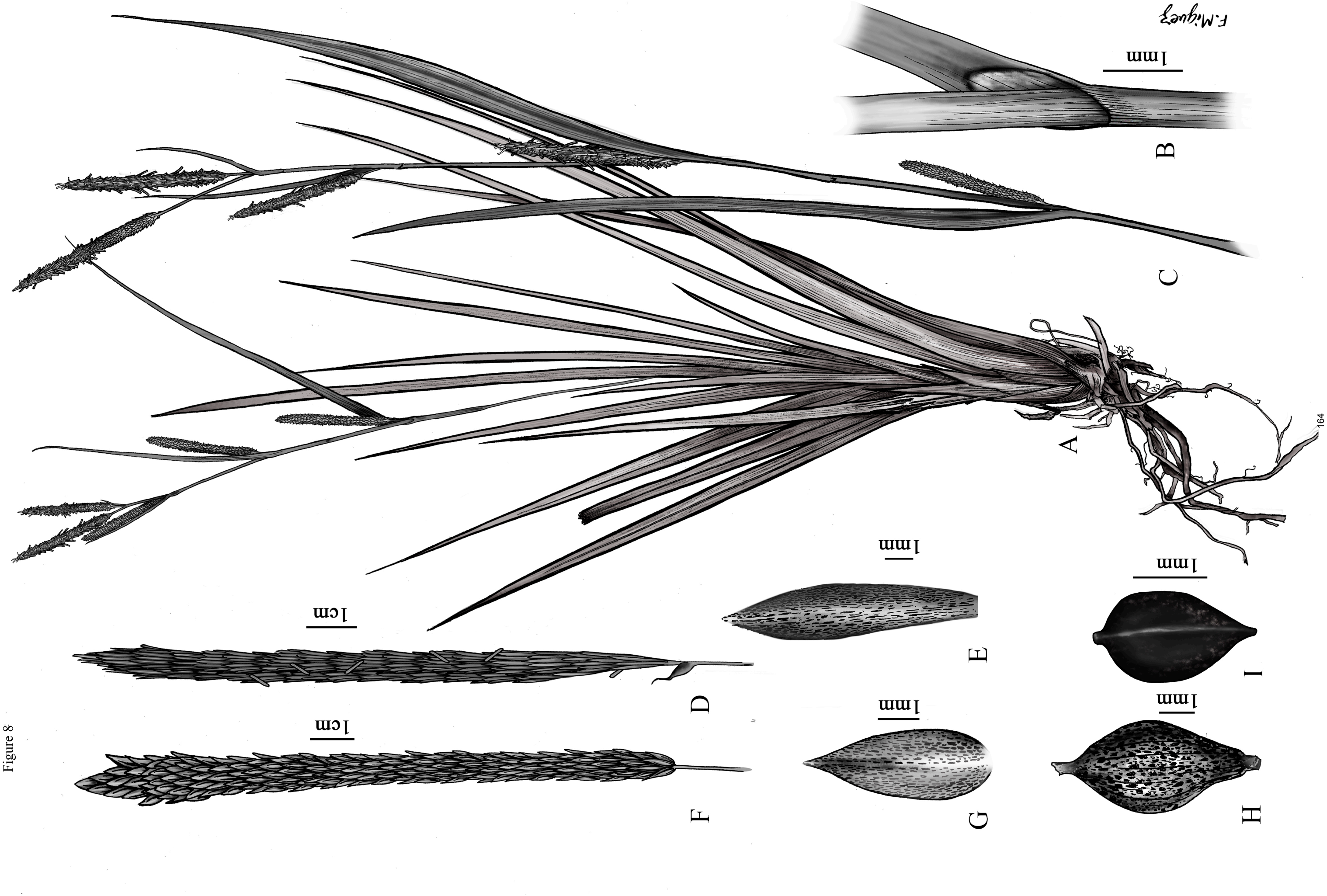


Figure 8

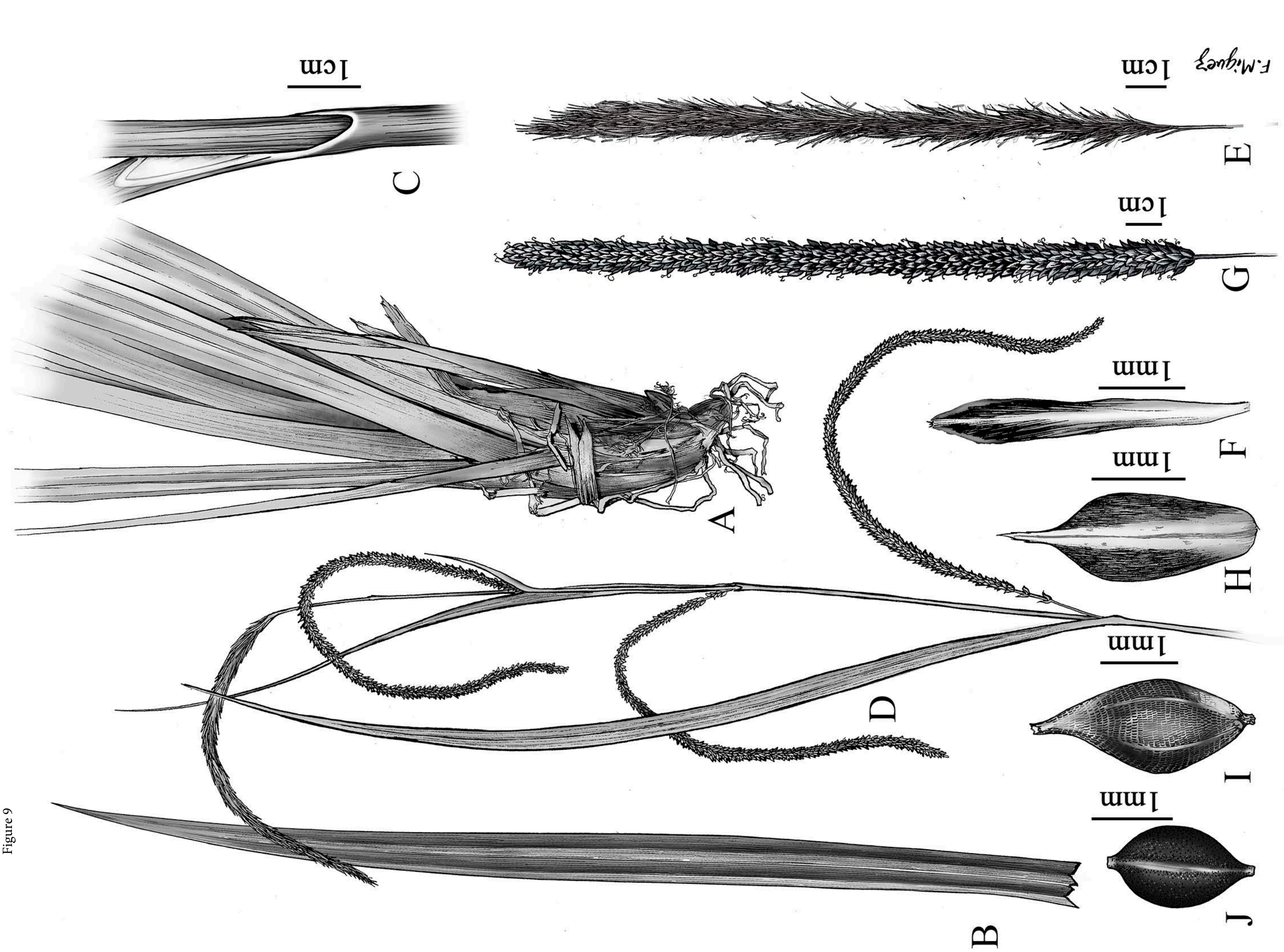
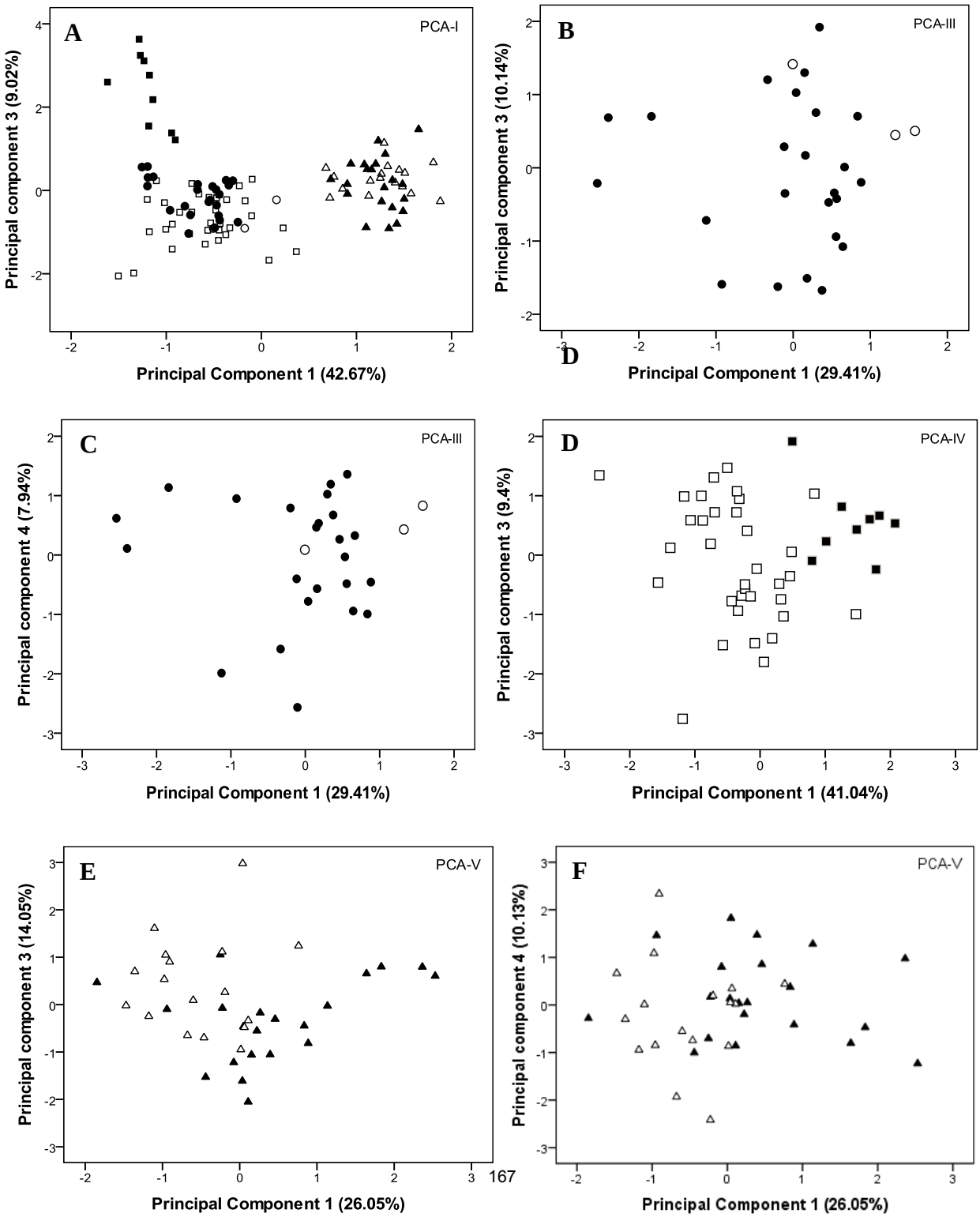


Figure 9

Figure 10

Supporting Information S1: Scatter plot of principal components extracted from the PCA as described in Figure 1



Supporting Information S2. Main results of the Discriminant Function Anlysis (DFA) resulting from *Carex* sect. *Rhynchocystis* (DFA-1) and *C. bequertii-C. mossii* (DFA-2) *datasets studied.*

DFA 1 (*C. agastachys, C. bequaertii-C. mossii, C. microcarpa, C. pendula, C. penduliformis*)

Lambda de Wilks

Functions	Wilks' Lambda	Chi-squared	df	Sig.
1 to 4	.001	350.525	120	.000
2 to 4	.023	198.962	87	.000
3 to 4	.099	121.353	56	.000
4	.327	58.690	27	.000

Correlations between discriminant variables and canonical discriminant functions from the DFA analysis *C. agastachys, C. bequaertii-C. mossii, C. microcarpa, C. pendula, C. penduliformis*

	Función			
	1	2	3	4
CLMW	.346	-.169	.268	.509
SLEAFW	-.144	.258	.682	.382
LIGL	.304	.136	.167	-.260
DLC	-.106	.104	-.610	-.037
PLC	.212	-.266	-.330	.379
SPKMN	**.731**	.258	-.210	-.097
SPKFN	.033	-.345	-.389	-.164
INFLF	-.153	**1.024**	-.363	-.262
INFWF	.063	.075	.450	.547
UL	**.654**	-.115	-.059	-.142
UMW	-.578	-.023	-.111	-.174
UBL	-.173	.735	.354	-.380
USL	-.284	-.116	-.361	**.683**
LUMWD	-.396	**.896**	.273	-.198
ACHL	-.438	-.379	**1.035**	-.423
ACHW	.101	.080	-.242	.214
ACL	.436	-.011	**-.713**	.321
ACHLACL	.594	-.667	-.364	.326
SSCLL	.561	-.064	-.407	-.559
SSCLW	-.143	.571	.352	-.196
SSCLL	**-.664**	**-1.091**	.086	-.146

SSCLW	-.019	.057	-.662	-.329
MAXCLPM	-.058	.091	.701	-.210
UPLDS	.504	.025	-.449	-.002
UPLMS	.439	.273	.362	.320
UPLPS	-.128	.416	.125	-.162
DSFS	.246	-.734	.373	.159
PSDFS	.504	.145	-.046	.377
FSLP	-.275	.761	.095	.142
PSPFS	.063	-.050	.025	.141

In bold, variables with the highest coefficients for each function.

Model validation

Groups' prior probabilities

Groups (1)*C. agastachys* (2)*C. bequaertii-mossii* (3)*C. microcarpa* (4)*C. pendula* (5)*C. penduliformis*	Priors	Selected original cases	
		Unweighed	Weighed
1	.200	11	11.000
2	.200	27	27.000
3	.200	6	6.000
4	.200	25	25.000
5	.200	2	2.000
Total	1.000	71	71.000

Classification table[a,b,d]

				Predicted group membership					
			Groups	1	2	3	4	5	Total
Cases selected	Original	Count	1	11	0	0	0	0	11
			2	0	27	0	0	0	27
			3	0	0	6	0	0	6
			4	2	0	0	23	0	25
			5	0	0	0	0	2	2
		%	1	100.0	.0	.0	.0	.0	100.0
			2	.0	100.0	.0	.0	.0	100.0
			3	.0	.0	100.0	.0	.0	100.0
			4	8.0	.0	.0	92.0	.0	100.0
			5	.0	.0	.0	.0	100.0	100.0
	Cross validation[c]	Count	1	9	0	1	1	0	11
			2	0	27	0	0	0	27
			3	0	0	6	0	0	6
			4	4	3	1	16	1	25
			5	0	0	0	0	2	2
		%	1	81.8	.0	9.1	9.1	.0	100.0
			2	.0	100.0	.0	.0	.0	100.0
			3	.0	.0	100.0	.0	.0	100.0
			4	16.0	12.0	4.0	64.0	4.0	100.0
			5	.0	.0	.0	.0	100.0	100.0

Cases no selected	Original	Count	1	6	0	0	0	0	6
			2	0	11	0	0	0	11
			3	0	0	3	0	0	3
			4	1	0	0	14	0	15
			5	0	0	0	0	1	1
		%	1	100.0	.0	.0	.0	.0	100.0
			2	.0	100.0	.0	.0	.0	100.0
			3	.0	.0	100.0	.0	.0	100.0
			4	6.7	.0	.0	93.3	.0	100.0
			5	.0	.0	.0	.0	100.0	100.0

a. 97,2% of selected original grouped cases correctly classified.

b. 97,2% of unselected original grouped cases correctly classified.

c.Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.caso.

d. 84,5% of selected cross-validated grouped cases correctly classified.

DFA 2 (*C. bequertii-C. mossii*)

Wilks' Lambda

Function	Wilks' Lambda	Chi-squared	df	Sig.
1	.237	23.787	13	.033

Correlations between discriminant variables and canonical discriminant functions from the DFA analysis *C. bequaertii* vs *C. mossii*

	Function
	1
DLC	**-1.047**
PLC	.907
UL	-.347
UMW	-.586
USL	.103
ACHL	-.617
ACHW	**1.225**
ACL	.305
SSCLL	-.292
UPLDS	.514
UPLMS	.428
UPLPS	**-1.103**

DSFS	.740

In bold, variables with the highest coefficients for the function.

Model validation

Groups' prior probabilities

Groups (1)*C. bequaertii* (2)*C. mossii*	Prioirs	Selected original cases	
		Unweighed	Weighed
1	.500	13	13.000
2	.500	12	12.000
Total	1.000	25	25.000

Classification table[a,b,d]

			Groups (1)*C. bequaertii* (2)*C. mossii*	Predicted group membership		
				1	2	Total
Cases selected	Original	Count	1	13	0	13
			2	1	11	12
		%	1	100.0	.0	100.0
			2	8.3	91.7	100.0
	Cross validation[c]	Count	1	7	6	13
			2	1	11	12
		%	1	53.8	46.2	100.0
			2	8.3	91.7	100.0

Cases no selected	Original	Count	1	5	2	7
			2	2	4	6
		%	1	71.4	28.6	100.0
			2	33.3	66.7	100.0

a. 96,0% of selected original grouped cases correctly classified.

b. 69,2% of unselected original grouped cases correctly classified.

c. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

d. 72,0% of selected cross-validated grouped cases correctly classified.

Chapter 5. Taxonomy of the Miocene relict giant sedges of *Carex* sect. *Rhynchocystis* (Cyperaceae) in Macaronesia, and description of the new species *Carex leviosa*

Taxonomy of the Miocene relict giant sedges of *Carex* sect. *Rhynchocystis* (Cyperaceae) in Macaronesia, and description of the new species *Carex leviosa*

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Abstract

Carex sect. *Rhynchocystis* (Cyperaceae) populations from the Macaronesian archipelagos (Azores and Madeira) have been considered traditionally either as a variety of the widely distributed Western Palearctic *C. pendula*, or directly synonymized with it. Recent phylogenetic studies, however, have shown that Azorean populations of *C. pendula* are phylogenetically distinct from continental populations. In this paper we perform a molecular phylogenetic analysis focused on the Macaronesian populations of *Carex* sect. *Rhynchocystis*, based on a comprehensive sampling and three nuclear DNA regions (*atpI-atpH*, ETS and ITS) regions plus two chloroplast DNA regions (*mat*K and *rpl*32-*trn*L^{UAG}). We combine the DNA analysis with a multivariate morphological analysis, to elucidate the phylogenetic relationships and taxonomic status of these island populations.

Our results suggest that the Azorean populations of *C. pendula* are better treated as an independent species within sect. *Rhynchocystis*, here described as *C. leviosa*. It constitutes the sister lineage to *C. pendula* and is also morphologically similar, but can be distinguished by its glumes, which are conspicuously longer than the utricles and pistillate glumes with a long scabrid awn. On the other hand, the populations of *C. pendula* from Madeira are not phylogenetically distinct from the continental populations. However, they show subtle morphological differences which, together with their geographical isolation, allow us to consider the Madeiran populations as *C. pendula* subsp. *myosuroides*.

Introduction

The Macaronesian archipelagos are a series of oceanic islands west of the European coast and North Africa, namely, the Azores, Madeira, the Canary Islands, and Cape Verdes.The flora of these archipelagos is rich in unique endemics, partly because they shelter a particular kind of cloud forest, the laurisilva. These lauroid forests have been long believed to be remnants of the vegetation that once covered Europe during the Cenozoic [1–3]. However, it has been confirmed that the majority of forest-forming species have a more-recent Plio-Pleistocene origin, so the laurisilva as species assemblage would not have such relict character [4,5]. On the contrary, a few studies show that at least some species would have an origin dating back to the Cenozoic, and so would be true relicts in the Macaronesian archipelagos [4–6].

Traditionally, Macaronesian taxa present in more than one archipelago have often been treated as conspecific [7]. The biogeographic patterns of plant diversity in the Azores that disconcerted Darwin were called 'Azores Diversity Enigma' by Carine & Schaefer (2010), and is characterized by the wide distribution of most native plant species across the archipelago, whereas few species are endemic of a single island. However, it has been shown that much of the apparent wide-spread endemics are groups undergoing allopatric speciation and on the way to multiple single-island endemics [9]. Also, there is growing evidence that populations from different archipelagos can be result from different colonization events from the Euro-Mediterranean region, thus representing different evolutionary lineages [10,11]. The Azores and Madeira archipelagos are part of the Mediterranean biodiversity hotspot [5], with some regions of high conservation interest [12] that are currently protected by the EU Habitat Directive [13].

Carex section *Rhynchocystis* (Cyperaceae) currently comprises five species of giant sedges, disjunctly distributed in the Western Palearctic (*C. agastachys* L.f, *C.*

microcarpa Bertol. ex Moris and *C. pendula* Huds.) and sub-Saharan Africa (*C. bequaertii* subsp. *bequaertii* De Wild. from West Africa, *C. bequaertii* subsp. *mossii* (Nelmes) Míguez, Gehrke, Martín-Bravo & Jim.-Mejías from South Africa, and *C. penduliformis* Cherm. from Madagascar). The systematics of the group have recently been studied based on both molecular and morphological data [14] and Míguez et al., under review). We found that section *Rhynchocystis* is a monophyletic group that diversified during the middle Miocene in Europe (Míguez et al. in press). Accordingly, the Western Palearctic species can be considered relict elements whose origin predates the onset of the Mediterranean climate [15]. *Carex pendula* has been reported to occur in Azores and Madeira and it has been treated as the same species occurring in Euro-Mediterranean Europe and North Africa. Kükenthal [16] accepted *C. pendula* var. *myosuroides* for the populations in the Azores and Madeira archipelagos. This variety was initially described as *C. myosuroides* by Lowe [17], based on plants from Madeira, an illegitimate name, being a later synonym of *C. myosuroides* Vill. [18]. In the first checklist of the Azorean flora [19], a Hochstetter specimen from Flores, Ribeira do Cruz is mentioned (coll. *Hochstetter 155* (TUB)), for which Seubert adopted the Lowe name. Later, Boott used Lowe's name [20] to base his *C. pendula* var. *myosuroides*. This variety name was used in most subsequent checklists for the Azores and Madeira [21,22]. Nonetheless, it has not been considered in later global treatments and checklists, which subsumed it under the synonymy of *C. pendula*[19, 21–23]. A recent phylogenetic study on *Carex* sect. *Rhynchocystis* [1] showed that *C. pendula* in its traditional circumscription actually included two distinct but hitherto overlooked lineages, *C. pendula* s.s., mainly from western Europe and northwestern Africa, and *C. agastachys*, mainly from eastern Europe and southwestern Asia. In this study the samples from Madeira and Azores grouped with *C. pendula* s.s. and thus were

considered to belong to this taxon. However, the two sampled populations from Azores constituted a well-supported clade sister to the rest of *C. pendula*, while the single sampled population from Madeira was placed at the base of *C. pendula* clade, but without significant support [14].

In this paper, we present a detailed reevaluation of the taxonomic status and relationships of the Macaronesian populations of *C. pendula*. We use DNA sequence data from two chloroplast (cp) DNA regions (*mat*K and *rpl*32-*trn*L^{UAG}) and three nuclear (nr) DNA regions (*atpI-atpH*, ETS and ITS), together with morphological trait data to elucidate (1) whether there are significant molecular/morphological differences between the Macaronesian plants and the continental *C. pendula* populations; and (2) if any of the population should be treated as a distinct taxonomic unit.

Materials and Methods

Molecular study

We tested the phylogenetic position of the Macaronesian populations by sampling one individual from five Azorean populations and a single sample from Madeira (Table 1). Eight samples of continental Euro-Mediterranean *C. pendula* from different populations covering its Western Palearctic range were also included, as well as six samples of *C. agastachys*, representing the genetic variation within lineage B of section *Rhynchocystis* (sensu Míguez et al. in press) As outgroup we included five species from other *Carex* sections (Table 1).

We sequenced and analysed two cp DNA (*mat*K and *rpl*32-*trn*L^{UAG}) and three nr DNA (*atpI-atpH*, ETS and ITS) regions, which have been successfully used in previous molecular systematic studies in *Carex* (i.e.Waterway & Starr, 2007; Escudero

& Luceño, 2009; Jiménez-Mejías et al., 2012; Maguilla et al., 2015). Most sequences were taken from the previous datasets [14]. We newly sequenced the five DNA regions for three Azorean populations (AZO3, AZO4 and AZO5) as well as the region *atpI-atpH* for six samples of *C. agastachys*, two Azorean populations (AZO1, AZO2), nine samples of *C. pendula*, and five outgroup samples (table 1), which adds up to a total of xx new sequences deposited in GenBank (acc. no. xx-xx).

atpI-atpH

Table 1 List of studied material. Sample label includes "botanical country" as in [31]. Collection data, voucher, ITS and GenBank accession numbers are provided.

Species (botanical country)	Country: locality	Voucher/herbarium code	*atpI-atpH* /ITS /ETS Accession number	*mat*K/ *rpl*32-*trn*L^{UAG} Accession number
Section *Rhynchocystis* Dumort.				
C. *agastachys* (AUT)	Austria, Vorarlberg.	*W. Lippert 15024* (M-0177708)	*atpI-atpH* ITS (KU939626) ETS (KU939551)	*mat*K (KU939705) *rpl*32-*trn*L (KU939780)
C. agastachys (CZE)	Czech Republic, Moravia Centralis.	*J. Dvorák s.n.* (M-0151978)	*atpI-atpH* ITS (KU939632) ETS (KU939557)	*mat*K (KU939711) *rpl*32-*trn*L (KU939787)
C. agastachys (GER)	Germany, Kreis Traunstein.	*W. Lippert* 23598 (M-0177733)	*atpI-atpH* ITS (KU939642) ETS (KU939564)	*mat*K (KU939720) *rpl*32-*trn*L (KU939797)
C. agastachys (UKR)	Ukraine, Veliky Berezny.	*A.K. Skvortsov s.n.* (M-	*atpI-atpH*	*mat*K (KU939729)

		0151973)	ITS (KU939650) ETS (KU939574)	*rpl*32-*trn*L (KU939806)
C. agastachys (YUG_SE)	Serbia, Bor District, Djerdap National Park.	*P. Jiménez-Mejías 86PJM10* (UPOS-4208)	*atpI-atpH* ITS (KU939660) ETS (KU939584)	*mat*K (KU939738) *rpl*32-*trn*L (KU939816)
C. agastachys(YUG_SL)	Slovenia, Podravksa, Ptju.	*M. Thulin s.n.* (UPS-V571925)	*atpI-atpH* ITS (KU939672) ETS (KU939597)	*mat*K (KU939750) *rpl*32-*trn*L (KU939828)
C. pendula (AZO 1)	Portugal, Azores, Pico Island.	*H. Schaefer 2013/89* (TUM)	*atpI-atpH* ITS (KU939628) ETS (KU939553)	*mat*K (KU939707) *rpl*32-*trn*L (KU939782)
C. pendula (AZO 2)	Portugal, Azores, Santa Maria Island.	*H.Schaefer 2013/90* (TUM)	*atpI-atpH* ITS (KU939629) ETS (KU939554)	*mat*K (KU939708) *rpl*32-*trn*L (KU939783)
C. pendula (AZO 3)	Portugal, Azores, São Miguel	*S. Martín-Bravo & L.*		

	Island.	*Bellón 136SMB15* (UPOS-6520(1/3))		
C. pendula (AZO 4)	Portugal, Azores, São Miguel Island.	*S. Martín-Bravo & L. Bellón 143SMB15* (UPOS-6830(1/7)).		
C. pendula (AZO 5)	Portugal, Azores, Faial Island.	*F.J. Salgueiro et al. 385* (SEV-275671)		
C. pendula (CYP)	Cyprus, Stavros-tis-Psokas.	*G. Alziar 0977* (SEV-251911)	*atpI-atpH* ITS (KU939631) ETS (KU939556)	*mat*K (KU939710) *rpl*32-*trn*L (KU939786)
C. pendula (DEN)	Denmark, Sydjylland.	*I. Segelberg s.n.* (V-181569)	*atpI-atpH* ITS (KU939633) ETS (KU939558)	*rpl*32-*trn*L (KU939787)
C. pendula (FRA)	France, Haute-Normandie, Eure.	*P. Jiménez-Mejías 15PJM10* (UPOS-4099)	*atpI-atpH* ITS (KU939634)	*mat*K (KU939712) *rpl*32-*trn*L

			ETS (KU939559)	(KU939789)
C .pendula (GER)	Germany, Baden-Württemberg, Heidelberg.	*K.P. Buttler 11244* (M-0177720)	*atpI-atpH* ITS (KU939644) ETS (KU939566)	*mat*K (KU939721) *rpl*32-*trn*L (KU939798)
C. pendula (GRB)	United Kingdom, London, Hampsted Heath.	*M.A. Spencer MAS/2012/040* (UPOS-5004)	*atpI-atpH* ITS (KU939648) ETS (KU939572)	*mat*K (KU939727) *rpl*32-*trn*L (KU939804)
C. pendula (ITA)	Italy, Torino.	*P. Jiménez-Mejías et al. 105bisPJM12* (UPOS)	*atpI-atpH* ITS (KU939653) ETS (KU939576)	*mat*K (KU939731) *rpl*32-*trn*L (KU939809)
C. pendula (MDR)	Portugal, Madeira, Santana.	*M.Sequeira MS7806 B,* (UPOS-5182)	*atpI-atpH* ITS (KU939656) ETS (KU939579)	*mat*K (KU939734) *rpl*32-*trn*L (KU939812)
C. pendula (MOR)	Morocco, Chefchaouèn.	*M. Ait Lafkih et al. 61,* (BM-340)	*atpI-atpH* ITS (KU939658)	*mat*K (KU939736) *rpl*32-*trn*L

			ETS (KU939582)	(KU939814)
C. pendula (SPA)	Spain, Jaén, Despeñaperros.	*P. Jiménez-Mejías 62PJM09* (UPOS-4720)	*atpI-atpH* ITS (KU939664) ETS (KU939588)	*mat*K (KU939741) *rpl*32-*trn*L (KU939820)
Section *Ceratocystis* Dumort.				
Carex flava L.				
C. flava	Norway, Laponia, Skjervoy.	*M. Luceño & M. Guzmán, 4005ML* (UPOS-403)	*atpI-atpH* ITS(AY278310) ETS(KU939525)	*mat*K(KU939681) *rpl*32-*trn*L (KU939760)
Section *Phacocystis* Dumort.				
Carex reuteriana Boiss.				
C. reuteriana	Spain, Cáceres.	*P. Jiménez-Mejías 57PJM07* (UPOS-5957)	*atpI-atpH* ITS(KU939602) ETS(KU939520)	*mat*K(KU939676) *rpl*32-*trn*L (JN222833)
Carex trinervis Dumort.				

C. trinervis	Spain, Huelva.	*P. Jiménez-Mejías 43PJM07*(UPOS-2205)	*atpI-atpH* ITS(KU939603) ETS(KU939521)	*mat*K(KU939677) *rpl*32-*trn*L (KU939756)
Section *Sylvatica* Huds.				
Carex sylvatica Huds.				
C. sylvatica	Switzerland, forest near Basel. (*atpI-atpH*, ITS, ETS, *rpl*32-*trn*L)	*Lechowicz s.n.,* (MTMG) (*atpI-atpH*, ITS, ETS, *rpl*32-*trn*L)	*atpI-atpH* ITS(AY757599) ETS(AY757660)	*rpl*32-*trn*L (KU939761)
	United Kingdom: Glamorgan. (*mat*K)	*G. Hutchinson, s.n.* (NMW175) (*mat*K)		*mat*K(JN896090)
C. rainbowii Luceño, Jim. Mejías, M. Escudero & Martín-Bravo				
C. rainbowii	South Africa: Kwazulu-Natal, Cathedral Peak Area.	*S. Martín-Bravo & M. Luceño 120SMB11* (UPOS-5030)	*atpI-atpH* ITS(KC122380) ETS(KC122388)	*mat*K(KU939682) *rpl*32-*trn*L (KU939762)

3

PCR conditions and primers followed those described in [32] for ITS, ETS and *matK*, and [33] for *atpI-atpH, and rpl32-trnL*^{uag}. PCR products were cleaned and sequenced as described in [14]. Raw sequences were edited, assembled, aligned and manually corrected also following [14]. IUPAC symbols were used to represent nucleotide ambiguities in ETS and ITS sequences. Congruence between nuclear and plastidial topologies was checked by eye and using Hompart test as implemented in PAUP* v. 4.0b10 (Swofford, 2002) in the Phylocluster (California Academy of Sciences). As no significant incongruences were found ($p>0.05$), we performed Bayesian Inference (BI) and Maximum Likelihood (ML) phylogenetic analyses on the concatenated matrix (*atpI-atpH* -ETS-ITS-*mat*K-*rpl*32-*trn*L^{UAG}) as performed in Maguilla et al. [30]. Substitution models were calculated for each DNA region separately in jModelTest v.2.1.3 [34] and selected based on the Akaike's Information Criterion weights (AICw [35]). Informative indels were coded as a fifth binary character state manually and analysed with the F81 model of sequence evolution as specified in MrBayes manual[36].

Morphological study

Macromorphological study

The morphological study included a total of 62 herbarium specimens from 12 herbaria (E, BM, K, LISU, M, MADJ, MHA, P, SEV, TUM, UPOS, UPS; S1 file; abbreviations following [33]) representing *C. agastachys* (23 specimens) and *C. pendula* (27 specimens), including six specimens from five populations from four different Azorean Islands (São Miguel, Faial, Pico and Santa Maria; S1 file) and six specimens from Madeira. Our sampling was designed to explore the morphological variation across the monophyletic group including *C. agastachys* and *C. pendula* (lineage B; see [1]).

Madeiran specimens from K (three; S1 file) were measured from high resolution digital images on-line available from the Global Plants Initiative hosted at JSTOR Global Plants (https://plants.jstor.org/), several variables of these samples could not be measured (PSDFS, PSPFS, SAP, SBL, SLI; see Table 2). In our morphological study, we considered 24 quantitative continuous (including one ratio), seven quantitative discrete and two qualitative characters (Table 2), mainly based on the characters used in our previous taxonomic revision of the section (Míguez et al., under review), plus four new characters based on our own observations (one quantitative continuous, LTPS (Lenght of the tip of the pistillate scale), and 3 quantitative discrete, SBL (Scabrousness of the basal leaf (measured in prickles per 0.25 cm^2), SAP (Scabrousness of the awn of the pistillate scale (number of prickles on the awn), SLI (Scabrousness of the lower bract of the inflorescence (measured in prickles per 0.25 cm^2)). Measurements were taken using an ocular micrometer, with the exception of the largest characters (more than 10 mm), which were measured using a standard 30-cm rule. The number of prickles on the female spike peduncle was counted on the 0.5 cm distal portion of the peduncles of the proximal and distal female spike. Two or three mature stems were measured per specimen and their averages included in the analyses.

Table 2. List of characters initially considered in the morphometric study.

Abbreviations	Continuous variables
ACHL	Achene length
ACHL/ACL	Ratio ACHL/ACL
ACHW	Achene width
ACL	Length from the achene base to the maximum width
CLMW	Culm width
DSFS	Distance between the two uppermost female spikes
FSLP	Peduncle length of the proximal-most female spike
INFLF	Female spike length
INFLM	Male spike length
INFWF	Female spike width
INFWM	Male spike width
LTPS	Lenght of the tip of the pistillate scale
LIGL	Ligule length
LUMWD	Length from the utricle base to its maximum width
MAXCLPM	Maximum length of the pistillate scale colored margin
PSGLL	Pistillate glume length
PSGLW	Pistillate glume maximum width
SLEAFW	Maximum leaf width
SSGLL	Staminate glume length
SSGLW	Maximum staminate glume width
UBL	Utricle beak length
UL	Utricle length
UMW	Utricle maximum width

USL	Utricle stalk length
	Discrete variables
SBL	Scabrousness of the basal leaf (measured in prickles per 0.25 cm^2)
SAP	Scabrousness of the awn of the pistillate scale (number of prickles in the bristle)
SLI	Scabrousness of the lower bract of the inflorescence (measured in prickles per 0.25 cm^2)
SPKFN	Female/Androgynous spike number
SPKMN	Entirely male spike number
PSDFS	Peduncle scabrousness of the distal female spike (number of prickles at the 0.5 cm distal portion of the peduncle)
PSPFS	Peduncle scabrousness of the proximal female spike (number of prickles at the 0.5 cm distal portion of the peduncle)
	Qualitative variables
DLC	Distal ligule color (0 whitish, 1 pale brownish, 2 reddish-purple)
PLC	Proximal ligule color (0 whitish, 1 pale brownish, 2 reddish-purple)

Principal Component Analysis (PCA) — For multivariate morphometric data we followed the sequential PCAs approach performed by [14] for section *Rhynchocystis* and inspired by [37] and [38]. Therefore, a first exploratory PCA with all 35 variables was conducted, which resulted on the retention of only those with the highest principal component (PC) loadings and higher correlation coefficients, although taking care of not including redundant variables. This character's purge implied the exclusion of variables with lower correlation scores and less PC contribution.

PCA-I was performed using ten variables (INFLF, INFLM, PLC, PSGLL, PSDFS, SAP, SLEAFW, SLI, SPKFN, UMW) and 62 specimens to explore the whole morphological dataset (*C. agastachys*, *C. pendula* from Azores, *C. pendula* from the Euro-Mediterranean region, *C. pendula* from Madeira).

PCA-II was performed using 11 variables (ACL, CLMW, DLC, INFLF, INFLM, FSLP, LUMWD, SPKFN, SSGLL, UL, UMW) and 32 specimens of *C. pendula* from the Euro-Mediterranean region vs. *C. pendula* from Madeira.

PCA-III was performed using five variables (LIGL, UBL, SAP, LTPS, SLI) and 12 specimens of *C. pendula* from Azores vs. *C. pendula* from Madeira.

Discriminant Function Analysis (DFA) — After the identification of homogeneous morphogroups, discriminant function analysis (DFA) was performed using the variables included in PCA-I, to assess taxonomic significant morphogroups as described in [38], considering as potentially significant those groups correctly classified for 80% of excluded cases as established in [37]. We randomly selected 70% of all samples to perform the DFA and for a cross-validation of the model over these samples. Then, the remaining 30% of the samples were randomly excluded from the analyses and used as a confirmatory blind control [37,38]. Results derived from the analyses implemented in IBM SPSS Statistics v.20 (IBM Inc., Chicago, IL, USA)

Mann-Whitney U test—As most of our data did not meet the assumption of normality required for the Shapiro-Wilk normality test, we used the Mann-Whitney U test to statistically validate the most discriminant characters differentiating between pairs of the species. This non-parametric test is analog to the two-sample t-test [39]. The level of significance was set at $P < 0.01$. These analyses were run in R (http://www.r-project.org/).

Micromorphological study

Micromorphology of the achene was examined under a Scanning Electron Microscope (SEM; GeminiSEM 300, Zeiss, Germany) following the same procedure as described in Miguez et al (under review). We applied this treatment to 13 achenes (five from Azores, three from Madeira, six from Euro-Mediterranean Europe). Two pictures with different zooms were taken from each sample (Fig 1).

Nomenclature

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to IPNI, from where they will be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix http://ipni.org/. The online version of this work is archived and available from the following digital repositories: PubMed Central, LOCKSS.

Results and discussion

Phylogenetic analyses

The aligned matrix of the concatenated *atpI-atpH* -ETS-ITS-*mat*K-*rpl*32-*trn*L^{UAG} DNA regions consisted of 24 sequences (Table 1) and 3560 aligned sites, four of which corresponded to coded indels. The nucleotide substitution model that best fit each DNA region based on jModelTest results were: HKY for *mat*K, GTR for *atpI-atpH* and *rpl*32-*trn*L^{UAG}, GTR + G for ETS and GTR+I+G for the ITS region. For the sake of simplicity we will discuss topological relationships on the basis of the topology recovered from the analysis of the nrDNA-ptDNA combined matrix (Fig 2). Bayesian inference and Maximum Likelihood analyses supported the monophyly of lineage B of *Carex* sect. *Rhynchocystis* with 1.0 posterior probability (PP) and 100% bootstrap support (BS), respectively (Fig 2). Section *Rhynchocystis* was strongly supported as sister group to representatives of section *Sylvaticae* (0.99 PP, 96% BS; Fig 2). *Carex pendula* was strongly supported as monophyletic and sister to *C. agastachys* (100% BS, 1.0 PP). The *Carex pendula* accessions grouped in two major clades (Fig. 2): Clade A (0.99 PP, 93% BS; Fig 2) includes only the Azorean samples and Clade B (0.93 PP; Fig 2) contains the rest of the populations studied, including the sample from Madeira.

Morphological study

Statistical analyses

In all datasets Kaiser´s measure of sampling adequacy was >0.5, and Barlett´s test of sphericity was significant. This implies that they are suitable to be explored using PCA [37,40]. Principal components extracted in each PCA are referred as PC and numbered using roman numerals.

Principal Component Analysis (PCA)

The numbers of samples in each analysis is different because it was not possible to measure all variables in all specimens.

PCA-I —We extracted three principal components (PCs) that accounted for 64.90% of the total variance (39.37%, 14.82%, and 10.71% respectively). The scatter-plot PC-1 vs PC-2 revealed a general structure with three clusters (Fig 3-A). The first cluster included samples of *C. pendula* from the Euro-Mediterranean region and from Madeira, the second cluster contained the samples from *C. agastachys*, and the third cluster contained the *C. pendula* samples from Azores. The characters that contributed the most to the first two components were INFLM, INFLF, SPKFN (Table 2). The scatter-plot PC-1 vs PC-3 did not retrieve clear separation between *C. agastachys* and Euro-Mediterranean *C. pendula*, but instead revealed a tendency of Madeiran populations to move away from the remaining samples (Fig 3-B).

PCA-II— We extracted three principal components (PCs) that accounted for 70.36% of the total variance (37.56%, 18.37%, and 14.42% respectively). The scatter-plot PC-1 vs PC-2 revealed the separation of populations of *C. pendula* from the Euro-Mediterranean region from those from Madeira (Fig 3-C).The scatter-plot and PC-1 vs PC-3 revealed a tendency of populations of *C. pendula* from Madeira to be separated from *C. pendula* of the Euro-Mediterranean region (Fig 3-D). The characters that contributed the most to the first two components were INFLF, UL, UMW.

PCA-III— We extracted two principal components (PCs) that accounted for 83.64% of the total variance (46.64% and 37%). The scatter-plots PC-1 vs PC-2 revealed the clear separation of Macaronesian *C. pendula* populations from Madeira from those from Azores (Fig 3-E). The characters that contributed the most to the first two components were SLI, UBL, LTPS.

Discriminant Function Analysis (DFA)

DFA— Once morphogroups were delimited by the subsequent PCAs procedure (see above), DFA analysis correctly classified 100% of the original selected cases and 90.5% in the cross-validation (S2 File). The analysis of unselected cases retrieved a 94.4% of cases correctly classified. Samples a priori belonging to *C. pendula* from Madeira were considered to belong to the Madeiran morphogroup in 72.7% of cases, whereas the remaining 11.1% were considered morphologically similar to the Azorean morphogroup (S3 File).

Despite some overlap in the range of many characters in all pairs of compared morphogroups, the Mann-Whitney U test retrieved significant differences (P-value<0.01) between *C. pendula* from Euro-mediterranean and *C. pendula* from Azores in five out of 31 quantitative characters (INFLM, LTPS, PSCLL, SAP, UMW; Table 2, S2 File) of which those that showed overlap of less than 25% were LTPS, PSGLL, SAP, and UMW. One character of *C. pendula* from the Euro-Mediterranean region and *C. pendula* from Madeira was retrieved by the Mann-Whitney U test with significant differences (P-value<0.01) (INFLF; Table 2, S2 File), while those that showed overlap of less than 25% between *C. pendula* from the Euro-Mediterranean and *C. pendula* from Madeira were INFLF, UMW. Boxplots of the most discriminant characters retrieved by DFA and also characters with less than 25% overlap are shown in Fig 4.

Discussion

An overlooked species of Carex pendula s.l. from the Azores

According to the recent phylogenetic and morphometric study by Míguez et al (under review), *Carex* section *Rhynchocystis* consists of five species. Three species are native to Europe (*C. agastachys, C. microcarpa* and *C. pendula*) and two to tropical Africa (*C. bequaertii, C. penduliformis*). Phylogenetically, *C. microcarpa* appears as sister clade of

the African species. *Carex agastachys* and *C. pendula* have an allopatric distribution and appear as sister species [1 and under review data]. Phylogenetic results (Fig 2) show that *C. pendula* actually includes two distinct well-supported lineages. These two lineages are allopatric, with a lineage (A) distributed across continental Europe, North Africa, the Mediterranean islands, and Madeira, and a second lineage (B) containing the samples from the Azores archipelago (Fig 2). The Madeiran samples are nested within *C. pendula* s.s, clustering together with samples from Cyprus and Morocco (but without support).

In addition to the phylogenetic separation between *C. pendula* from the Euro-Mediterranean and the Azores populations, significant morphological differences are found. PCA clearly separated samples from both population groups (Fig. 3-A), five out of the 31 measured characters presented significant differences between both groups based on Mann-Whitney U test and four characters showed overlap of less than 25 (see results and Fig. 4). Populations from Azores present a congruent and distinct set of clear-cut morphological characters that do not match the typical morphology of the other species of section *Rhynchocystis* in general, and specifically of Euro-mediterranean populations of *C. pendula*. In particular, these plants are display pistillate glumes with a long scabrid awn 0.4-1mm, among others differences (see Table 3 and diagnosis), which readily allow their distinction from other species in the section. The Madeiran plants, despite being nested within *C. pendula* in the molecular phylogeny estimate (Fig. 2), display a particular morphology characterized by at least one feature significantly different from the rest of the studied *C. pendula* populations: the female spike length (LTPS), which is larger in the Madeiran plants (131)187-210(260)mm (Fig 4, Table 3). The distinctiveness of the Madeiran populations is additionally supported by the synapomorphic combination of characters including most lateral spikes

androgynous, with a short conical tip containing male flowers, and pistillate and staminate glumes bearing scabrid awns.

In our opinion, such findings support the distinctiveness of Azorean plants from *C. pendula* s.s., and its consideration as a different species (*Carex leviosa*, described below) endemic to this Atlantic archipelago. On the contrary, the fact that the Madeiran plants are already morphologically distinct, but not yet in analyzed DNA regions points to a more recent disjunction of these plants. Also, it cannot be discarded the maintenance of certain degree of gene flow between the archipelago and the continent, which is much closer to mainland than the more isolated Azores islands. Accordingly, we consider the Madeiran populations an extreme of variation within *C. pendula*, a fact that is emphasized by their geographical separation (morphogeographic compartimentalization [41], and we suggest that these plants should be considered as a subspecies of *C. pendula* (subsp. *myosuroides*).

The *C. pendula* plants from Azores and Madeira has been sometimes considered as *C. pendula* var. *myosuroides* [42]. We found that the Azorean and Madeiran plants are different biological entities. The molecular data do not only point to a clear differentiation between the two groups of Macaronesian populations, but also show that Madeiran plants are more closely related to *C. pendula* from the continent than the Azorean plants are (Fig 2). These results are compatible with two independent colonization events to the archipelagos from the western Palearctic, as a previous ancestral area reconstruction also suggested [1]. Similarly, the Macaronesian species *Carex lowei* Bech (Madeira), *C. hochstetteriana* Gay (Azores) and *C. perrauderiana* Gay (Canarias) which are closely related and belong to the same section (Spirostachyae), have been found to have evolved independently in each of the three

Macaronesian archipelagos, and were suggested to have colonized each of them in a different long-distance dispersal event [43].

Taxonomic treatment

The following treatment accounts for the variation of *Carex* section *Rhynchocystis* in the Macaronesian archipelagos of Azores (*C. leviosa*) and Madeira (*C. pendula* subsp. *myosuroides*). Since the newly considered taxa are split from a formerly more widely conceived *C. pendula*, we also included a description of the more narrowly circumscriped *C. pendula* subsp. *pendula* excluding the variation of the Macaronesian populations. Terminology for prophylls follows [44].

Carex leviosa Míguez, Jim.-Mejías & Martín-Bravo, sp. nov. (Fig 5)

- *Carex myosuroides* auct., non Lowe, Trans. Cambridge Philos. Soc. 4(1): 10 (1833), nom. illeg.

- *Carex pendula* auct., non Huds., Fl. Angl.: 352 (1762)

[urn:lsid:ipni.org:names:XXXXXXXXXX]

Diagnosis – Previously considered as part of the morphological variability of *C. pendula* Huds. The new species differs from *C. pendula* by the glumes conspicuously longer than the utricles, Glume body 0.4-4 mm longer than utriculum, (instead of glumes equaling or shorter than the utricles)(Table 3), pistillate glumes longly awned, 0.4-1mm, with scabrid awn and hyaline margin (instead of pistillate glumes acute or mucronate (Table 3); glabrous in all of its surface and without hyaline margin), and utricles and achenes slightly smaller. Also *C. leviosa* has the male spike and the uppermost female spike clustered at the apex (instead 1(2) male spikes at the apex, not

clustered with a female spike). See Table 3 for a detailed comparison of diagnostic characters to distinguish *C. leviosa* and the two *C. pendula* subspecies.

Table 3. Diagnosis characters distinguishing *C. pendula* subsp. *pendula* from its relatives *C. pendula* subsp. *myosuroides* and *C. leviosa*

	C. pendula		***C. leviosa***
	subsp. *pendula*	**subsp. *myosuroides***	
Peduncle of lowermost spike	60–90 mm, smooth or sparsely scabrid	40–160 mm, usually smooth	(10)75–150 mm, sparsely scabrid
Inflorescence	Inflorescence with 1(2) male spikes at the apex, and (4)6–7 lateral female spikes, rarely shortly androgynous	Inflorescence with 1 male spike at the apex, and 4–6(8) lateral androgynous spikes	Inflorescence with 1 male spike and 1 female spike clustered at the apex, and 5–6 lateral female spikes
Lateral Spike length	85-170 mm	(131)187-210(260)mm	120-170mm
Pistillate glume	Body of the pistillate glume equaling or shorter than the utricle. Pistillate and staminate glumes acute or mucronate, without hyaline	Body of the pistillate glume generally shorter than the utricles or shortly surpassing them. Pistillate glume and pistillate and staminate glumes	Body of the pistillate glume conspicuously longer than the utricles. Pistillate and staminate glumes long awned, with hyaline margins.

	margin.	usually with scabrid awn; without hyaline margin.	
Lenght of the tip of the pistillate glume (LTPS)	0-0.5mm	0.4-0.7mm	0.4-1mm
Utricle	(1.4)2-3.6 × (0.5) 0.96–1.5 mm	1.4–2.7 × 0.6–0.8 mm	2.5–2.86 × (0.7)0.9–1(1.1)mm
Achene	Elliptical (1)1.3–1.8(2.1) × (0.4)1.4–1.5 mm	Elliptical 1.3–1.7 × 0.5–2 mm	Narrowly oblong (1.1)1.4–1.7 × 0.7–0.9 mm

2

Type –PORTUGAL: Azores archipelago, São Miguel Island, NW of the Island, between Mosteiros and Pilar de Bretanha, João Bom, about 500 meters before the village; dry stream in ravine, humid and shady understory in *Pittosporum* forest with many introduced species. 26 August 2015. S. Martín-Bravo & L. Bellón (136SMB15). (Holotype, UPOS-6520; isotypes, MA, MADJ).

Description – Rhizome densely cespitose. Stems 150-250 cm × 2–4 mm, smooth or -slightly scabrid towards the apex. Leaf blades 13–20 mm wide; ligule 20-35 mm long, purple-reddish, longer than wide, acute or emarginate; basal sheaths entire scale-like, reddish-brown. Inflorescence racemose with 1 male spike and 1 female spike clustered at the apex, and 6–7 lateral female spikes entirely female or more rarely very shortly androgynous; lowermost bract leaf-like, slightly shorter than the inflorescence. Male spike (75)120–185 mm × 2–5 mm, fusiform, pendulous, sessile.

Lateral spikes 120–170 mm × 4–7 mm, long cylindrical, pendulous, distal ones with the peduncle progressively shorter, proximal-most one long-pedunculate, peduncle (10)75–150 mm long, sparsely scabrid, all with a tubular cladoprophyll at the base.

. Staminate glumes 3.4–7.1 mm × 0.7–1.1 linear, oblong or narrowly obovate, brownish with a midrib prolonged in a scabrid awn; pistillate glumes 3.4–5.8(7) × 0.7–1.2 mm including the awn, the body longer than the utricles, narrowly ovate to narrowly obovate, brownish with hyaline margins and with a pale midrib prolonged in a scabrid awn 0.4-1 mm. Utricles 2.5–2.9 mm × (0.7) 0.9–1(1.1) mm, ellipsoid, greenish to brown at maturity, gradually attenuated into a beak 0.2–0.5 mm, truncated. Achenes (1.1) 1.4–1.7 mm × 0.7–0.9 mm, narrowly oblong, brownish.

Distribution and habitat –AZO. Endemic to the Azores archipelago (Portugal), where it is distributed in all nine main islands (Corvo, Faial, Flores, Graciosa, Pico, Santa

Maria, Terceira, and São Miguel, São Jorge). It is a common plant on Flores and Faial and scattered on Santa Maria [43]. Shady humid lauroid forest understorey, mainly in *Pittosporum* and laurel forests, ravines and craters. 0-800 m.a.s.l.

Phenology – Flowering and fructification occur from July to September.

Etymology – The epithet was proposed by Paula and Raquel, Mónica Míguez's daughters. It refers to the magic levitation spell "Wingardium Leviosa", from J. K. Rowling's Harry Potter universe, as the robust thick lateral spikes of *C. leviosa* hang from thin, long peduncles, looking like they are levitating in the air. The resulting construction parallels the meaning of *C. pendula*, 'hanging'. The selected epithet intends to commemorate Paula and Raquel and at the same time, we want the epithet also to serve as a tribute to J. K. Rowling, because of her dedication and concern about nature and wildlife, as expressed in her work "Fantastic Beasts and where to find them" [45].

Carex pendula* subsp. *myosuroides (Boott) Míguez, Martín-Bravo & Jim. Mejías stat. et comb. nov. Fig. 6 (I, J).

[urn:lsid:ipni.org:names:XXXXXXXXXX]

≡*Carex pendula* var. *myosuroides* Boott, Ill. Gen. Carex 4: 197 (1867) [basionym]

Lectotype here designated: PORTUGAL: Madeira archipelago, Madeira Island, North side of Madeira by rivulets. 1837. J. Boott, s.n. (Lectotype, K000363417 digital image!)

Additional collections (paratype) — PORTUGAL North side of Madeira by rivulets. 31-08-1837. J. Boott 653(K000363419 (K, digital image!).

≡ *Carex myosuroides* Lowe, Trans. Cambridge Philos. Soc. 4(1): 10 (1833), nom. illeg.

Ind. Loc.: "Hab. in Maderae ora septentrionali" [Madeira, Portugal]

*Description***–** Rhizome densely cespitose. Stems 50–180(240) cm × 2–2.8 mm, smooth. Leaf blades 13–15 mm wide; ligule (16)21–23(32) mm long, whitish, hyaline, becoming brownish when dry, longer than wide, acute to subacute; basal sheaths scale-like, reddish. Inflorescence with 1 male spikes at the apex, and 4–6(8) lateral female spikes finished in a conical tip containing male flowers; lowermost bract leaf-like, equaling or slightly shorter than the inflorescence. Male spikes 65–260 × 4–5(8) mm, long-cylindrical finished in a conical tip pendulous, subsessile. Lateral spikes (131)187–210 (260) × 4–5(8) mm, long-cylindrical, flexuose, spreading or pendulous, shortly androgynous, distal spikes sessile or subsessile,; proximal-most one long-pedunculate, peduncle 40–160 mm, usually smooth, all with a tubular cladoprophyll at the base. Staminate glumes (3.4) 4.6–6.3 × 0.35–0.9 mm linear, oblong or narrowly obovate, brownish with a midrib prolonged in a scabrid awn; Pistillate glumes 2.3–2.7(3.7) × 0.7–0.8 mm including the awn, the body shorter than the utricles or shortly surpassing them, narrowly ovate to narrowly obovate, reddish-brown with a greenish midrib prolonged in a scabrid awn 0.4-0.7 mm. Utricles 1.4–2.7 × 0.6–0.8 mm, ellipsoid, greenish, red dotted, beak 0.1–0.3 mm, truncated. Achenes 1.3–1.7 × 0.5–2 mm, elliptical, brownish to yellowish.

Distribution and habitat – MDR. Edges of streams

Phenology – Flowering and fructification occur from April to August.

Etymology – *myosuroides*, from *myos*, Ancient Greek for mouse, and *uros*, Ancient Greek for tail, 'resembling a mouse tail', perhaps because the lateral spikes are gradually tapered at the tip into a thinner male segment, somewhat resembling a tail.

Notes: The selected neotype for the illegitimate name *C. myosuroides* Lowe corresponds with the author's taxonomic concept of this name, as reflected by the annotations made by Lowe and sent to Boott.

Carex pendula* subsp. *pendula Huds, Fl. Angl.: 352 (1762). Fig. 6 (A-H).

Ind. Loc – "Habitat in sylvis et sepibus humidis; in sepibus inter Hampstead et Highgate copiose" [England, United Kingdom].

Neotype – Morison, 1699, Pl. Hist. Univ. Oxon. 3m sect. 8, tab. 12, fig. 4. designated by Egorova (1999). *Epitype*: London. Hampstead Heath, between Hampstead and Highgate, Ken Wood lake –vc 21, Middlesex, *M. A. Spencer* MAS-2012-040 (BM001074530!), designated by Jiménez-Mejías et al. (in press); *isoepitype*: UPOS!

Description **–** Rhizome densely cespitose. Stems 50–180(240) cm × 2–6 mm, smooth or slightly scabrid distally. Leaf blades 6–16 mm wide; ligule 12–37 mm long, whitish, hyaline, becoming brownish when dry, rarely slightly reddish-tinged, acute to subacute; basal sheaths faint, scale-like, dark brown or reddish, often the stem bases covered by old-leaf remnants, pale brown. Inflorescence with 1(2) male spikes at the apex, and (4) 6–7 lateral female spikes, exceptionally shortly androgynous; lowermost bract leaf-like, equaling or slightly shorter than the inflorescence. Male spikes (20)70–130 × 2.5–9 mm, fusiform or cylindrical, erect, spreading, or pendulous, sessile or subsessile, sometimes with a peduncle up to 2.5 cm. Lateral spikes 85–170 × 3–6.5(8) mm, long-cylindrical, flexuose, spreading or pendulous, subsessile or with peduncles 60–90 mm, usually smooth, rarely sparsely scabrid, all with a tubular cladoprophyll at the base. Staminate glumes 3.6–8.7(9.8) × 0.2–1.2(1.9) mm, linear, oblong or narrowly obovate, acute, reddish-brown with a hyaline midrib; Pistillate glumes 2.2–3.1(3.9) × (0.7)0.8–1(1.2) mm, the body generally

shorter than the utricles or shortly surpassing them, narrowly ovate to narrowly obovate, reddish-brown with a greenish midrib, acute to shorlty mucronate, the mucro 0-0.5 mm long, smooth. Utricles (1.4)1.96–3.6 × (0.5)1.1–1.5 mm, ovoid or ellipsoid, greenish or yellowish green, beak 0.2–0.5 mm, truncated. Achenes (1)1.3–1.8(2.1) × (0.4)1.4–1.5 mm, elliptical, with the maximum width at the middle or slightly above it, brownish to yellowish. 2n=58 [22,46–49].

Distribution and *habitat* – Europe and the Mediterranean, including northwestern Africa and the Mediterranean shores of southwestern Asia, introduced in southern Scandinavia, and confirmed also as introduced in New Zealand and North America [ALG BGM COR CYP DEN EAI FRA GER GRB GRC BEL DEN HUN IRE IRQ? ITA KRI LBS MOR NET nzs ore PAL? POR SAR SIC SPA swe SWI TUN TUR YUG_CR YUG_MN YUG_SL]. Riparian forests, beside streams, usually on damp clayish soils. 25–1370 m.a.s.l.

Phenology– (March) April–August(December).

Etymology– From the Latin *pendulus*, hanging, in reference to the pendulous lateral spikes.

KEY TO THE SPECIES OF *CAREX* SECT. *RHYNCHOCYSTIS*

1. Body of the pistillate glume conspicuously longer than utricles … 2

1´. Body of the pistillate glume shorter to equaling or shortly surpassing the utricles … 3

2 . 1 Uppermost spike entirely male; lateral spikes usually female, rarely androgynous; achenes narrowly oblong; ligule reddish…***C. leviosa***

2’. Uppermost spikes with male and female flowers, very rarely entirely male; lateral spikes always with some male flowers intermingled between the female ones; achenes obovate to elliptic; ligule whitish … ***C. bequaertii***

2.a. Pistillate glumes brown, usually with a wide middle central nerve lighter than the scale sides; achenes (1)1.4–1.9(2.1) × (0.3)0.7–0.9 mm; ligule subacute, sometimes emarginate … ***C. bequaertii*** subsp. ***bequaertii***

2.b. Pistillate glumes pale brown, with a ± narrow middle nerve usually darker than the scale sides; achenes 1.4–1.8 × 0.8–1mm; ligule emarginate … ***C. bequaertii*** subsp. ***moosii***

3. All spikes erect or the lowermost one slightly spreading, subsessile or the lowermost with a peduncle up to 50 mm; leaves strongly coriaceous; ligule from acute to obtuse; stems 40–100 cm tall … ***C. microcarpa***

3´. At least the lowermost spike conspicuously pendulous when mature, with a peduncle (0)25–100(160) mm; leaves herbaceous, soft, non-coriaceous; ligule acute or subacute; stems usually more than (50)100 cm tall … 4

4. Uppermost 3–6 spikes sessile, male and female ones separated by very short internodes, appearing like an aggregate cluster of spikes tipping the inflorescence; mature utricles and achenes dark-brown to blackish … ***C. penduliformis.***

4´. Uppermost spikes pedunculate, with apparent internodes between them, occasionally only the 2 uppermost male spikes (when more than 1) closely arranged; mature utricles and achenes greenish, yellowish, or light-brown … 5

5. Achenes obovate, with the widest point near the top; peduncle of the lowermost spike conspicuously scabrid; ligule reddish; utricle beak bidentate or truncate … ***C. agastachys***

5´. Achenes elliptic, with the widest point at the middle or slightly above it; ligule

whitish becoming brownish when dry, rarely slightly reddish-tinged; peduncle of the lowermost spike smooth or sparsely scabrid; utricle beak truncate … ***C. pendula***

5´.a. Pistillate glumes with a scabrid awn 0.4-0.7 mm; lateral spikes (131)187–210(260) mm, androgynous, with a conical tip containing the male flowers … ***C. pendula*** subsp. ***myosuroides***

5´.b. Pistillate glumes acute or mucronate, with a short smooth mucro, 0-0.5 mm; lateral spikes 85–170 mm, female, rarely shortly androgynous … ***C. pendula*** subsp. ***pendula***

Acknowledgements

The authors thank to all staff from herbaria E, BM, K, LISU, M, MADJ, MHA, P, SEV, TUM, UPOS, UPS for granting us access to their collections and for providing plant material, M. Sequeira (Universidade da Madeira) for providing plant material; E. Maguilla and M. Luceño (Universidad Pablo de Olavide) for advise during the preparation of the manuscript, F. Míguez for the illustrations of specimens; the staff of UPOS herbarium and Pablo de Olavide University microscopy service, in particular to F. J Fernández and F. Rodriguez Rojas, for technical support. This research was supported by the Ministry of Economy, Industry and Competitiveness through the project CGL2012-38744.

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Supporting Information

S1 File. Specimens included in the morphometric and/or molecular analysis.

S2 File. Discriminant function analysis (DFA) results using morphological variables measured in *Carex pendula* s.l. Variables included: INFLF, INFLM, PLC, PSGLL, PSDFS, SAP, SLEAFW, SLI, SPKFN, UMW.

Figure leyends

Figure 1. Scanning electron micrographs of the entire achene (A, B, C; scale bar 100 μl) and detail of the achene surface (D, E, F; scale bar 10 μl) in *C. pendula* subsp *pendula* (A, D); *C. pendula* subsp. *myosuroides* (B, D); *C. leviosa* (C,F).

Figure 2. Majority-rule consensus tree of *Carex* sect. *Rhynchocystis* inferred under Bayesian inference using the combined nrDNA-ptDNA matrix (*atpI-atpH*, ETS, ITS, *mat*K and *rpl*32-*trn*L^{UAG} regions). Numbers above and below the branches indicate clade support values: Maximum Likelihood and Bayesian posterior probability, respectively. Tip labels indicate species names and codes of the source regions (in parenthesis), following "botanical countries" as in Brummitt (2001), and including a number when there is more than one sample from the same region. Scale bar indicates substitutions per site.

Figure 3. Scatter plot of the first principal components extracted from the PCA. A and B (PCA-I)-*C. agastachys*, *C. pendula* from Euro-mediterranean , *C. pendula* from Azores, *C. pendula* from Madeira; C and D (PCAII)- C. *pendula*, *C.pendula* from Madeira; E and F(PCA III)- *C. pendula* from Azores and *C. pendula* from Madeira. Symbols depict the different species or population set considered: *C. agastachys* =white triangle, *C. pendula*=white square, *C. pendula* from Azores =black circle, *C. pendula* from Madeira = black triangles.

Figure 4. Boxplots of the most discriminant characters retrieved by DFA or with less than 25% overlap. The X-axis represents the considered species or population set labelled as follows: AGA (*C. agastachys*), AZO (*C. pendula* from Azores), MAD (*C. pendula* from Madeira), PEN (*C. pendula*). The boxes cover 50% of the data values

ranging between the 25th and 75th percentiles, and the lines show 90% of the values between the fifth and 95th percentiles. The line within the box represents the median. Outlying values are indicated by small "o's" and far out values are indicated by asterisks (*)

Figure 5. Analytical illustration of the holotype of *Carex leviosa* Míguez, Jim.-Mejías & Martín-Bravo. Azores San Miguel, NW of the island, between Mosteiros and Pilar de Bretanha. 26 August 2015. S. Martín-Bravo & L. Bellón 136SMB15 (UPOS-6520). (A) Culm base; (B) ligule (C); inflorescence; (D) male and female spike (E)pistillate glume ; (F); staminate glume (G) utricle; (H) achene.. Drawing by F. Míguez.

Figure 6 Analytical llustration of *Carex pendula* subsp *pendula*. (A, B, D, F, H) Italy, Tuscany. 13 June 2010. P. Jiménez-Mejías 24PJM10, UPOS-4136; (F, H) France, Cévennes national Park. 25 June 2009. P. Jiménez-Mejías 104PJM09, UPOS-5878; (C, E, G) Italy, Piedmont, 8 June 2012. P. Jiménez-Mejías & E. Martinetto 64PJM12, UPOS-5348. *Carex pendula* subsp. *myosuroides* (I, J) Portugal, Madeira. 21 July 1987. Nobrega MADJ.05494. (A) ligule; (B) inflorescence; (C); male spike (D); staminate glume; (E) female spike; (F) pistillate glume; (G) utricle; (H) achene; (I) lateral spike of *C. pendula* subsp. *myosuroides*; (J) pistillate glume of *C. pendula* subsp. *myosuroides*. Drawing by F. Míguez.

Figure 1

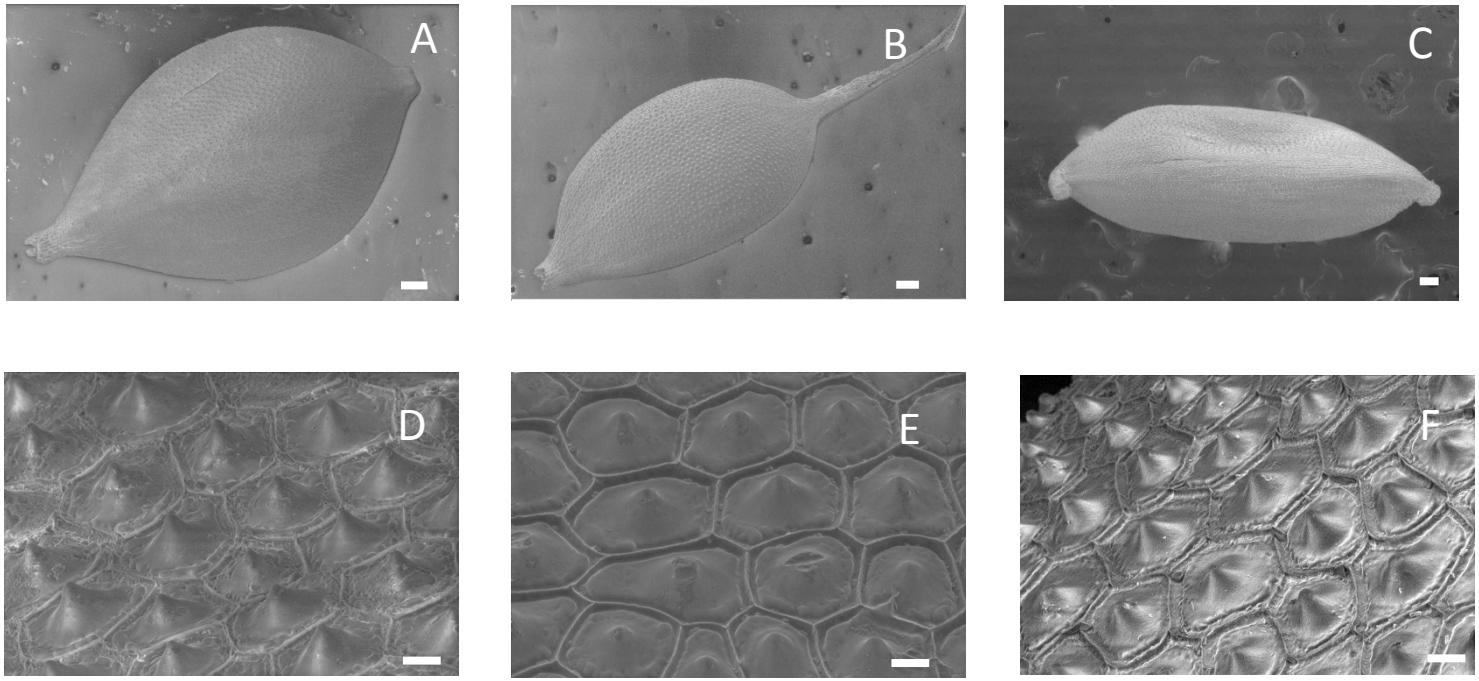


Figure 2

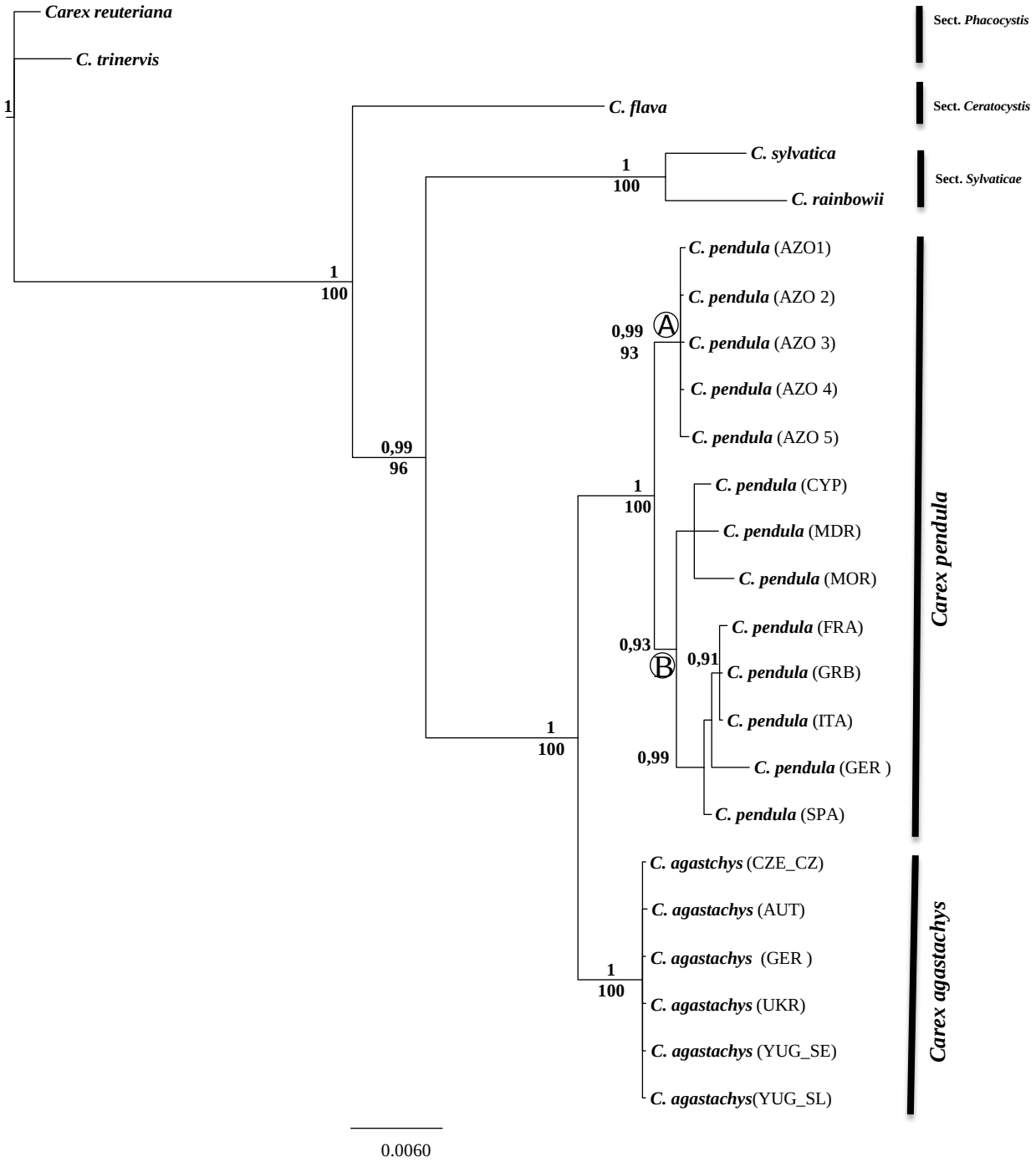

Carex reuteriana
C. trinervis
C. flava
C. sylvatica
C. rainbowii
C. pendula (AZO1)
C. pendula (AZO 2)
C. pendula (AZO 3)
C. pendula (AZO 4)
C. pendula (AZO 5)
C. pendula (CYP)
C. pendula (MDR)
C. pendula (MOR)
C. pendula (FRA)
C. pendula (GRB)
C. pendula (ITA)
C. pendula (GER)
C. pendula (SPA)
C. agastchys (CZE_CZ)
C. agastachys (AUT)
C. agastachys (GER)
C. agastachys (UKR)
C. agastachys (YUG_SE)
C. agastachys(YUG_SL)
1
1
100
0,99
96
1
100
0,99
93
A
0,93
B
0,91
1
100
0,99
1
100
Sect. Phacocystis
Sect. Ceratocystis
Sect. Sylvaticae
Carex pendula
Carex agastachys
0.0060

Figure 3

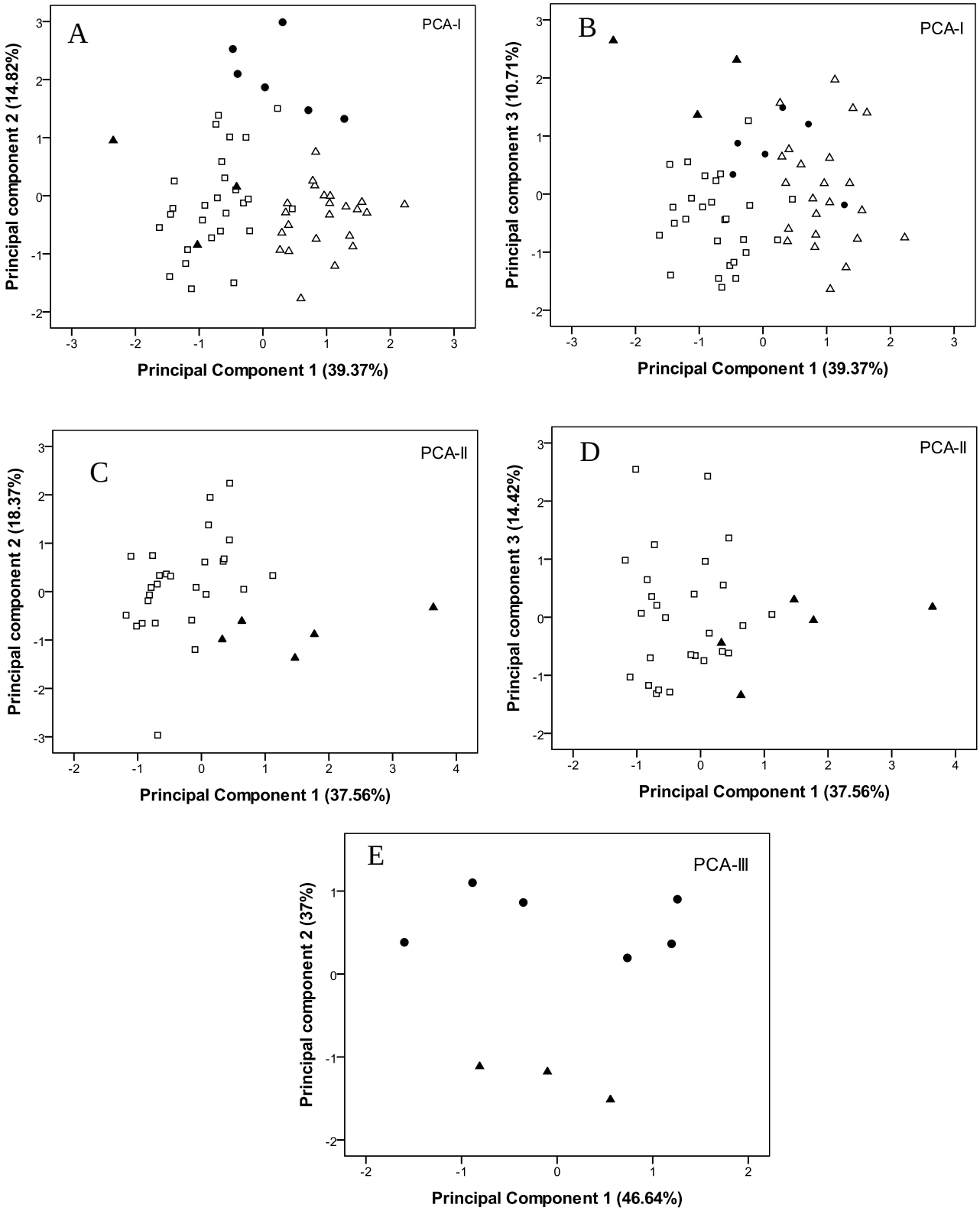
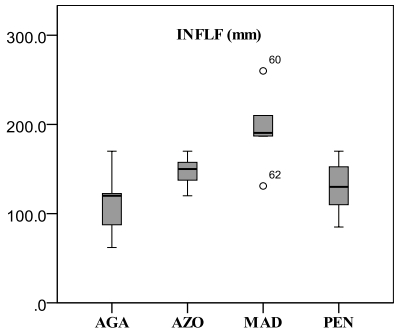
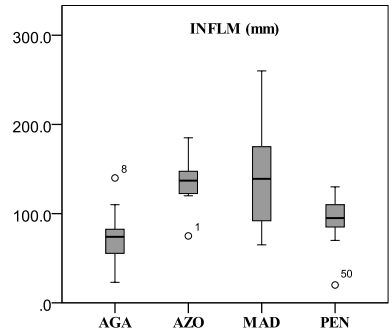

A
PCA-I
Principal component 2 (14.82%)
Principal Component 1 (39.37%)
B
PCA-I
Principal component 3 (10.71%)
Principal Component 1 (39.37%)
C
PCA-II
Principal component 2 (18.37%)
Principal Component 1 (37.56%)
D
PCA-II
Principal component 3 (14.42%)
Principal Component 1 (37.56%)
E
PCA-III
Principal component 2 (37%)
Principal Component 1 (46.64%)

Figure 4


INFLF (mm)
300.0
200.0
100.0
.0
60
62
AGA
AZO
MAD
PEN


INFLM (mm)
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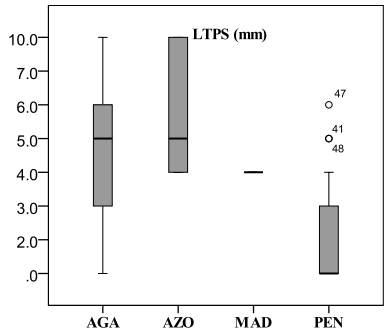
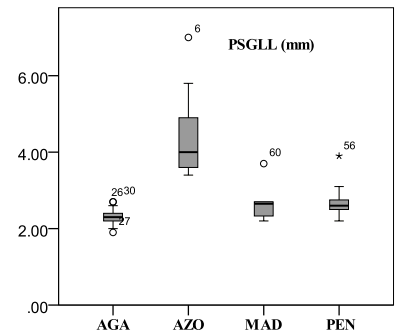
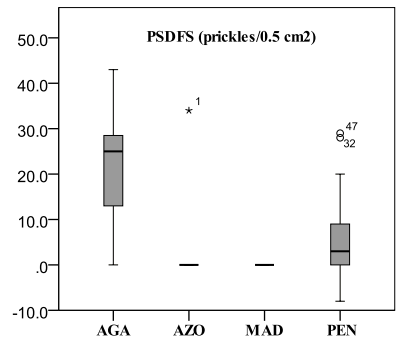
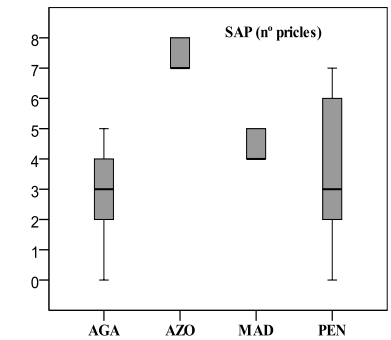
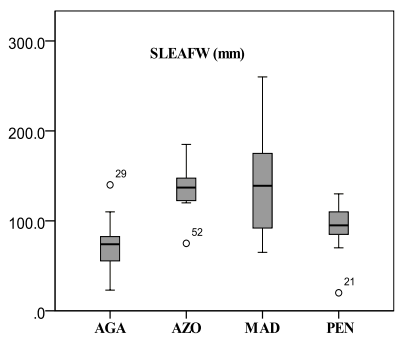
200.0
100.0
.0
8
1
50
AGA
AZO
MAD
PEN


LTPS (mm)
10.0
7.0
6.0
5.0
4.0
3.0
2.0
.0
47
41
48
AGA
AZO
MAD
PEN


PSGLL (mm)
6.00
4.00
2.00
.00
6
60
56
26
30
27
AGA
AZO
MAD
PEN


PSDFS (prickles/0.5 cm2)
50.0
40.0
30.0
20.0
10.0
.0
-10.0
1
47
32
AGA
AZO
MAD
PEN


SAP (nº pricles)
8
7
6
5
4
3
2
1
0
AGA
AZO
MAD
PEN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100.0
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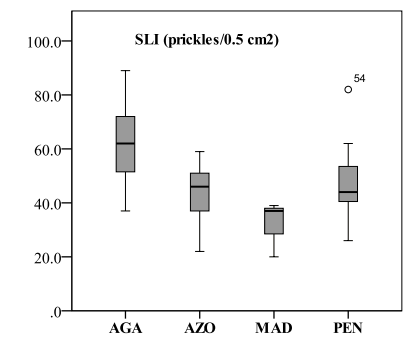
29
52
21
AGA
AZO
MAD
PEN


SLI (prickles/0.5 cm2)
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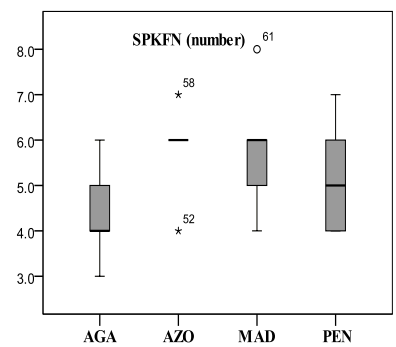
60.0
40.0
20.0
.0
54
AGA
AZO
MAD
PEN


SPKFN (number)
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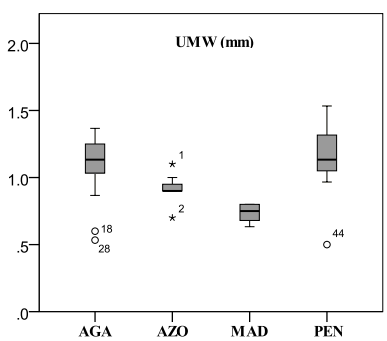
7.0
6.0
5.0
4.0
3.0
61
58
52
AGA
AZO
MAD
PEN


UMW (mm)
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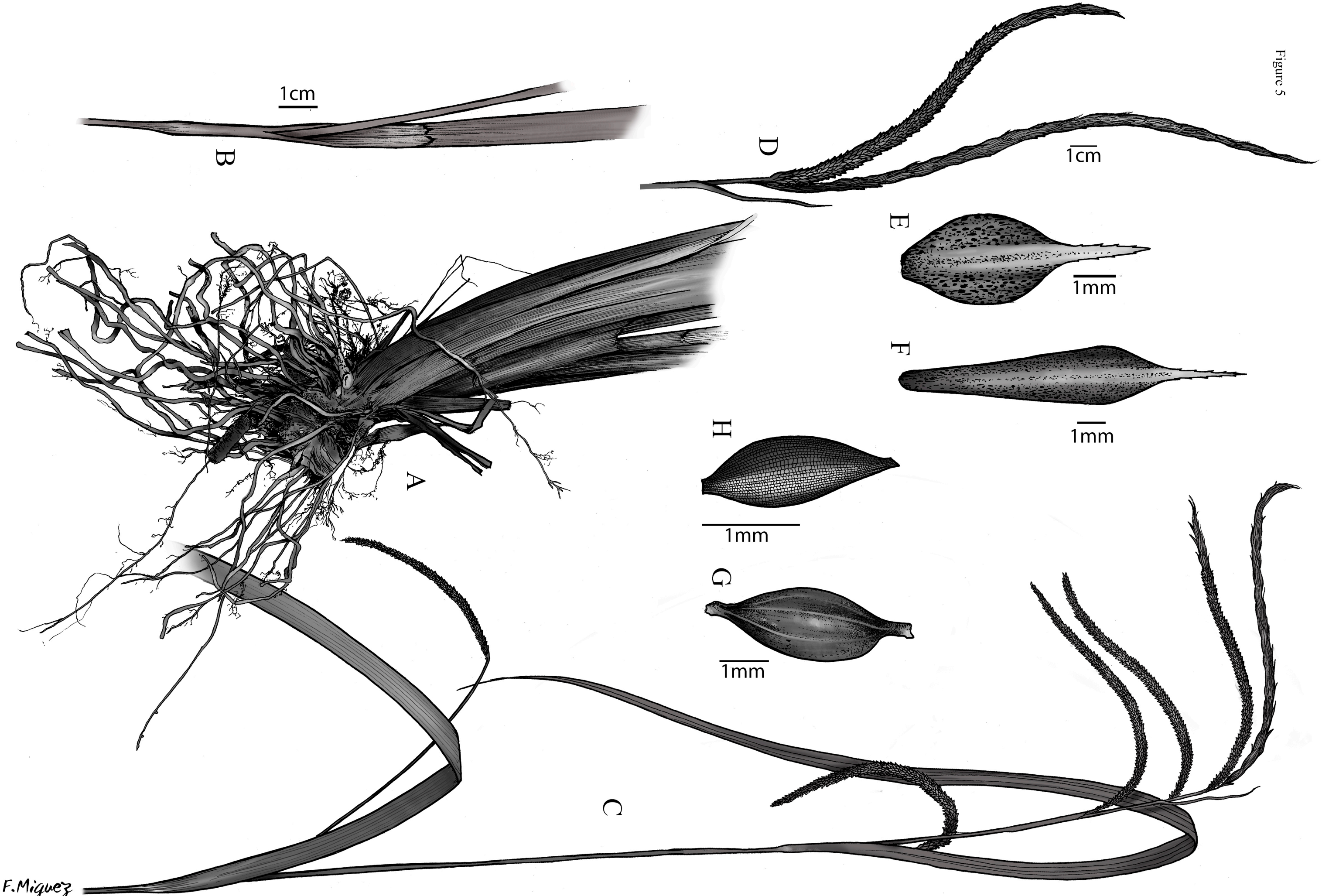
1.0
.5
.0
1
2
18
28
44
AGA
AZO
MAD
PEN

Figure 5



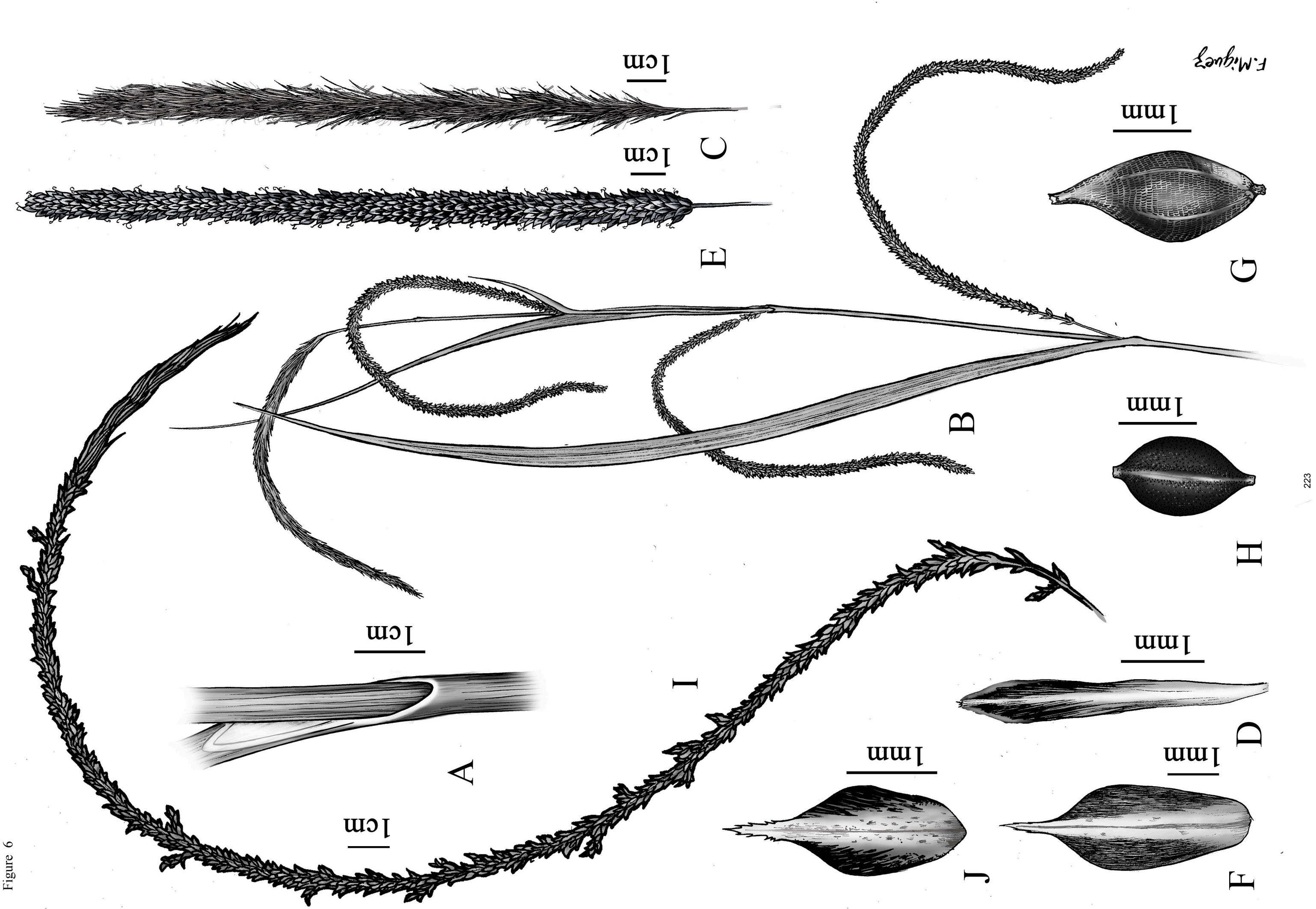


Figure 6

Supporting Information

S1 File. Specimens examined. Bold = specimens included in the morphometric and/or molecular ♦ analysis.

Carex agastachys

AUSTRIA: ♦**Vorarlberg, 25 June 1974, W. Lippert 15024 (M-0177708)**; Tirol, Wallchsee-Kranzach, 26 Aug 1968, W. Lippert 166563. (M-0177709); Attersee area, village Unterach, SW of Stockwinkel, 28 May 1996, E.Vitek 96-244 (M-0177712); Baden-Württemberg, Heidelberg, 2 June 1984, Fr. Hörl s.n. (M-0177726); Bavaria, Kreis Freising; St. Alban, 11 August 1980, J.Sellma MTB 7837/3 (M-0177729); Village Unterach am Attersee, SW of Stockwinkel, 28 May 1996, E. Vitek 96-244 (UPS-V149677). AZERBAIJAN: Mesazhai, 7 May 1991, N.V.Kostylëva & N.V.Kostyleva, s.n. (MHA); Lekoransky Zone. Alekseyevka, 27 May 58, V.G.Eropob s.n. (MHA). BULGARIA: Burgas, M. StrandZa, N. Vihodcevsky s.n. (UPS-V571923). CZECH REPUBLIC: ♦**Moravia Centralis, 10 Jun 1962, J. Dvorák, s.n. (M-0151978).** GERMANY: Oberbayern, Kreis Traunstein; Ruhpolding, 22 June 1903, (M-0177732); Bayern, Kreis Unterallgäu, 1 July 1984, W. Lippert 20008 (M-0177736); Kreis Traunstein, 12 June 1988, W . Lippert 23598 (M-0177733). IRAN: Gilan, Navrud (Asalem) to Herowabad, 15 May1971, J. Lamond 2967 (E-00305405); Golestan, Bender Ges: near Wonischtepe, 24 March 1901, P. Sintenis 1455 (UPS-V571920). SERBIA: ♦**Bor District, Djerdap National Park. Miroc Mountains, 21 June 2010, P. Jiménez-Mejías 86PJM10 (UPOS-4208)**. HUNGARY: a Csikos to Mellett, 12 May 1971, G. Sag 530 (P-02208912); SLOVAKIA: Pukanec, Bakabánya, 1901, S. Kupeok 27308 (M-0177702); Australis, distr. Banska Stiavnica, June 1898, S. Kupcok, s.n. (P-

02231326). SLOVENIA: ♦**Ljubljana, Pekel-Schlucht südlich Borovnica, 28 May 1966, K.P. Buttler 9566 (M-0177703)**; ♦ **Podravksa, Ptju, 9 May 1972, M. Thulin 1974 (UPS-V 571925)**; TURKEY: Trabzon, Trabzon-Macka, 1 July 1974, Ansin, R 1476 (E-00449228). UKRAINE: ♦**Veliky Berezny, 12 July 1968, A.K.Skvortsov s.n. (M-0151973)**.

Carex pendula

ALGERIA: Kabylie de Collo, bords des ruisselets, dans la basse vallée de l'Oued Tamanart, près du littoral, 26 May 1944, L. Faurel s.n. (P-01998578). BELGIUM: Liège, Seraing, 19 June 1999, J. Lambinon 99/248 (M-0177701). CROATIA: Licko-senjska: Dalmatien, PlitviCke, 28 June 1938, H. Lenander s.n. (UPS-V571926). CYPRUS: ♦**Environs de Stavros-tis-Psokas, 21 April 1991, G. Alziar 0977 (SEV-251911)**. CZECH REPUBLIC: Zlínský, Vlára, 29 July 1932, J.PodpÊra 994 (UPS-V571922). DENMARK: Bornholm d.47 Vertermarie, Almindingen, 15 June 1977, P. Lütken s.n. (P-01889138); ♦**Sydjylland, Distr. 52 22 Jun 1949, I. Segelberg (UPS-V181569)**. FRANCE: ♦ **Haute Normandie, 8 May 2010, P. Jiménez & J.M. Toro 15PJM10 (UPOS-4099)**; Cevennes, road between Les plantiers and Valleraugue, 25 June 2009, P. Jiménez_Mejías 104PJM09 (UPOS-5878). GERMANY: ♦**Baden-Württemberg, Heidelberg, K.P. Buttler 11244 (M-0177720)**; Saarbrücken, 13 June 1910, Ruppert s.n. (M-0177723); Bayern, Thalkirchen, Golfplatz, 5 July 1991, H. Förther 4868 (M-0177739). GREAT BRITAIN: **London, Hampsted, Heath, Lake Ken Wood, 5 August 2012, M.A. Spencer, MAS/2012/040 (UPOS-5004)**; E. Sussex, Fairlight Glen, near Hastings, C. Bailey 1459 (M-0177699). GREECE: Attica, pr. Baphi, 10 May 1931, F.G.Guiol 1869 (UPS-V571924). ITALIA: Elba, Marciana Marina, 9 May 1986, Laubwald s.n. (M-0177704); Tuscany, alpi apuane, Vinca, 13

June 2010, P. Jiménez-Mejías 24PJM10 (UPOS-4136). ITALY: **Torino, Puente de Valle Ceppi, June 2012, P.Jiménez-Mejías et al., 105bisPJM12 (UPOS)**. LUXEMBOURG: Sauergebiet, 25 May 1926, W. Freiberg 1459 (M-0177697). MOROCCO: **Chefchaouèn, 3 June 2005, M. Ait Lafkih et al., 61 (BM-340).** PORTUGAL: Alentejo, Mora, May 2014, R. Palhinha & F. Mendez P6 (LISU-7404); SPAIN: Cáceres, Navalconcejo, 7 May 2011, P. Jiménez-Mejías 52PJM11 (UPOS-4719); ♦**Jaén, Despeñaperros, Aldeaquemada, 24 May 2009, P. Jiménez-Mejías 62PJM09 (UPOS-4720)**; Asturias, Colunga. Playa de la Griega, 5 August 2012, F.J.Fernández 10FJF12 (2/2) (UPOS-4973); Cáceres, Cascadas Nogaleras, 11 May 2012, M. Maguilla 4EMS12BIS (UPOS-5143); Sevilla, Cazalla de la Sierra; Parque Natural Sierra Norte Sevilla. 27 September 2003, P. Jiménez-Mejías 61PJM03 (UPOS-5877); Huelva, Cueva de la Mora, June 2004, J.M.Marín 904JMM 1/2 (UPOS-953); Huelva, Cueva de la Mora, June 2004, J.M.Marín 904JMM 2/2 (UPOS-954). SWITZERLAND: Lugano, Mendrisio, 6 June 1963, J. Höller s.n. (M-0177707); Ginebra, Terre de Prégnu, pré de fauche, 24 May 2001, R. Piñeiro_Portela s.n. (UPOS-2649).

Carex pendula population from Azores Islands

♦**Faial, Cabeço do Fogo, 20 June 2011, Salgueiro et al. 157/11(SEV 275671)**; ♦**Cova do Cabo da Canada, 12 Sept. 2013, Schaefer 2013/89 (TUM)**; ♦**Santa Maria, São Lourenço, 10 Sept. 2013, Schaefer 2013/90 (TUM)**; ♦ **São Miguel, between Mosteiros and Pilar de Bretanha, 26 Aug. 2015, Martin-Bravo & Bellon 136SMB15(1/3) (UPOS-6520(1/3))**; S**ão** Miguel, between Mosteiros and Pilar de Bretanha, 26 Aug 2015, Martin-Bravo & Bellon 136SMB15(2/3) (UPOS-6520(2/3)); S**ão** Miguel, between Mosteiros y Pilar de Bretanha, 26 Aug 2015, Martin-Bravo &

Bellon 136SMB15(3/3) (UPOS-6520(3/3)); ♦**São Miguel, Lagoa das Furnas, 27Aug 2015, Martin-Bravo & Bellon 143SMB15(1/7) UPOS-6830(1/7)**;

Carex pendula populations from Madeira

♦**Santana, Dic 2013, M. Sequeira, MS7806 B (UPOS-5182)**; Levada da Torre, 21 Jul 1987, Nobrega 273 (MADJ-05494); Ribeiro Bonito, 13 Jul 1985, Nobrega (MADJ-05492); Ribeira da Ametade, 14 Aug 1849, Lowe 639 (K-00036418); North side of Madeira by rivulets, 31 Aug 1837, Boott 653 (K-000363419); North Madeira by rivulets, 1837, Boott, s.n. (K000363417).

Outgroup

Carex flava L. Norway, Laponia, Skjervoy, 6 August 2005, M. Luceño & M.Guzmán, 4005ML (UPOS-403).

C. rainbowii Luceño, Jim. Mejías, M. Escudero & Martín-Bravo. South Africa: Kwazulu-Natal, Cathedral Peak Area, Rainbow Gorge, 13 November 2011, S. Martin-Bravo & M. Luceño, 120SMB11 (UPOS-5030).

Carex reuteriana Boiss. Spain, Cáceres, May-June 2007, P. Jiménez-Mejías, 57PJM07 (UPOS-6957).

Carex sylvatica Huds. Switzerland, forest near Basel, Lechowicz, *s.n.* (MTMG)(*atpI-atpH*,ITS, ETS, *rpl*32-*trn*L); United Kingdom: Glamorgan. NMW175 (*mat*K).

Carex trinervis Dumort. Spain, Huelva, 27 April 2007, P. Jiménez-Mejías 43PJM07 (UPOS-2205)

Chapter 6. General discussion and conclusions

Discussion

For the present PhD project, we have integrated different kind of data (morphology, DNA sequences, fossil record, biogeography) aiming for an integrative study of *Carex* section *Rhynchocystis*. This discussion has been structured in two parts. In the first one, the taxonomic arrangement of section *Rhynchocystis* is discussed at the light of the molecular and morphological data in an evolutionary context. The second part addresses the evolutionary processes that could have been involved in shaping the biogeographic patterns of the section, including ancestral areas and the evaluation of processes of long-distance dispersal vs. vicariance.

1. A new, systematic treatment of the Carex section Rhynchocystis based on an integrated, molecular and morphological study

The section *Rhynchocystis* within the genus *Carex*

Our phylogenetic results provide a new systematic framework for *Carex* section *Rhynchocystis* (see chapters 2 and 3), which serves as the basis of a new, more natural, taxonomic classification. *Carex* section *Rhynchocystis* is placed within *Core Carex* (Global Carex Group, 2016; see introduction Fig. 1) placed together with sections *Sylvaticae*, *Ceratocystis*, *Spirostachyae* and *Rostrales*. Our results partially corroborate those obtained by other authors (see Fig. 3 in introduction; [1–3] regarding the relationship of *Rhynchocystis* with other sections. Thus, *Carex* section *Rhynchocystis* is grouped together with the section *Sylvaticae* in those cladograms when combined nuclear and plastidial markers (Chapter 2, Fig. 3 and Chapter 5, Fig. 2) or only with nuclear markers (Figs. S1, S2 and S3 in Appendix 2 of Chapter 2) while *Rhynchocystis* is nested together with the remaining sections if only plastid data is analyzed (Fig. S4 in Appendix 2 of Chapter 2). The incongruences in tree topology performed with different

molecular markers might be caused by hybridization or incomplete lineage sorting [4–7]. Although both processes have been described in the genus *Carex* [8–11], lineage sorting is perhaps the most probable at such not sallow phylogenetic level [12,13].

A combination of characters is needed to define section *Rhynchocystis* because it lacks unique morphological autoapomorphies. This has been previously retrieved in other *Carex* sections (reviewed in GCG 2016). Thus, plants within section *Rhyncocystis* are large herbs that can reach up to 3 meters in height, sharply trigonous stems and leaves flat to M-shaped in cross section, ligule present and basal sheaths scale-like. Inflorescences racemose, with 5–8 narrowly cylindrical spikes. The 1(2) uppermost one(s) erect or slightly arching, usually entirely male the 2–8 lateral ones usually very long and pendulous entirely female or very shortly androgynous, all with a tubular cladoprophyll at the base. Pistillate glumes, mucronate or awned. Stigmas 3. Utricles elliptic or ovate, obtusely and usually asymmetrically trigonous, smooth, glabrous. Achenes elliptic, obovate or ovate, trigonous, smaller than bodies of perigynia (see chapter 4 for complete description of the section).

Species within *Carex* section *Rhynchocystis*

The new taxonomic scheme proposed for section *Rhynchocystis* reflects the evolutionary history of organisms [1] and ensures nomenclatural stability keeping those taxa whose identity is well established in the literature and / or are easily recognizable morphologically [14]. Different concepts of species have been applied to the great variety of taxonomic groups to accurately fit their most important biological properties [15]. The taxonomic circumscription we perform in our revision of *Carex* section *Rhynchocystis* has been done based on two species concepts: 1- phylogenetic species, it is, to fulfill monophyly; we used the molecular data to search for monophyletic groups.

And 2- morphological species, to find morphological autoapomorphies for the considered taxa (chapters 4 and 5). In addition, the species delimitation we performed within section *Rhynchocystis* also fit into the wider concept of cohesive species, since we addressed our study from an evolutionary perspective [16]. The concept of cohesive species entails a combination of the phylogenetic, biological and taxonomic species concepts that takes into account evolutionary history, reproductive isolation, and ecology. The cohesive concept is important because re-establishes the important role of natural selection, already advanced by Darwin, in speciation.

The initial taxonomic scheme of the section considered five species: *C. bequaertii*, *C, microcarpa*, *C. mosii, C. pendula* and *C. penduliformis*. Our first phylogeny revealed that of these 5 species *C. microcarpa* and *C. penduliformis* corresponded with monophyletic groups, and *C. bequaertii* and *C. mossii* were not mutually monophyletic. The morphometric analyses, however, revealed six homogeneous morphogroups (chapters 4 and 5) that were monophyletics (chapters 2 and 5), although they did not exactly mach the five traditionally retrieved species: *C. pendula* was divided in two distinctive morphogroups, whereas *C. bequaertii* and *C. mossii* were recovered as a single morphogroup.

Among the taxonomic results obtained in this thesis, it is remarkable that only *C. microcarpa* and *C. penduliformis* were supported in their traditional concepts. *Carex pendula* was revealed to be composed of two strongly differentiated lineages that were also found to be morphologically distinct. These two lineages were recognized at the species level in congruence with its phylogenetic and morphological differentiation (see Chapters 2, 4 and 5). *Carex agastachys* L.f. is a cryptic species that have been hidden under the synonymy of its sister species *C. pendula* s.s. , during more than two centuries. Both taxa had remained unnoticed despite the clear-cut differences probably

because their mostly allopatric distribution drawn a fake continuous distribution, misleading the botanist to consider them as a single continuously-distributed species. Our morphological study showed than the most diagnostic morphological characters distinguishing between *C. agastachys* and *C. pendula* were achene shape, color of the ligule and scabrousnness of the peduncle. Characters traditionally used to distinguish species within Section *Rhynchocystis*, such as utricle or achene size ([17], appeared to be homoplasious for these two sister species (see chapter 3).

In contrast, *C. bequaertii* and *C. mossii* were not differentiated in our phylogenetic study (Chapter 2) and only very subtly in our morphological evaluation (Chapter 3).It has been controversial to use the degree of geographic isolation to divide morphologically poorly defined populations in range of species or below [18,19]. We have used the infraspecific criteria proposed by [18] where disjunt distribution is used as additional information to consider them as two different subspecies. Thus, taking into account the phylogeny, the biogeographic history (see below) and the morphometric study of *C. bequaertii* and *C. mossii*, we consider them as a single species with two subspecies disjunctly distributed: *C. bequaertii* subsp. *bequaertii* and *C. bequaertii* subsp. *mossii.*

Further analyses focusing on Macaronesian plants revealed the existence of an additional monophyletic group sister to *C. pendula.* The addition of another plastid region to the phylogeny identified *C. leviosa* endemic to Azorean archipelago as a phylogenetic species distinct from *C. pendula*. *Carex leviosa* has also been hidden under *C. pendula*. This taxon also meets the requirement of being a morphological species, being recovered as a homogeneous morphogroup in our morphometric analyses (chapter 5). Although the morphometric study showed that specimens from Madeira have morphological differences by means of this new phylogeny, they just constituted

an extreme of variation within *C. pendula*. This fact is emphasized by their geographical separation, led us to considered the Madeiran populations as *C. pendula* subsp. *myosuroides*.

2 Biogeographic patterns

2.1 Disjunctions in *Carex* sect. *Rhynchocystis.*

Disjunct distributions, where the same or closely related taxa are found in areas separated by clear geographic barriers, are one of the most intriguing patterns in biogeography. The origins of plant disjunctions have been historically explained under three alternative evolutionary scenarios: parallel evolution, vicariance, and long-distance dispersal [5,20–23]. *Carex* sect. *Rhynchocystis* displays an interesting pattern of disjunction (chapter 2-Fig. 1). Species are distributed either in the western Palearctic (temperate Europe and Mediterranean basin -*C. agastachys*, *C. microcarpa*, *C. pendula* subsp. *pendula*-, Macaronesia -*C. leviosa*, *C. pendula* subsp. *myosuroides*-), and in sub-Saharan Africa (*C. bequaertii* subsp. *bequaertii*, *C. bequaertii* subsp. *mossii*, *C. penduliformis*) (see Fig.1 in chapter 2). Interestingly, similar distribution patterns are also displayed by other closely related sections in the genus: *Spirostachyae* [8,24], *Ceratocystis* [25], and *Sylvaticae* [3].

Ancestral area estimation, together with phylogenetic and dating analyses (see chapters 2 and 5) have provided important information to infer the historical events that could have shaped the disjunct distribution of *Rhynchocystis*. The origin of the most recent common ancestor (mrca) of *Carex* section *Rhynchocystis* was estimated to have taken place around 23.07 Ma (28.6-18.36 Ma at 95% highest posterior density interval, HPD), probably in southwestern Europe–northwestern Africa (Chapter 2). Such old age,

together with its continuous fossil record from the Miocene to the present, makes section *Rhynchocystis* one of the few examples of Tertiary-origin relict groups in the Western Mediterranean.

Lineage evolution and distribution of *Carex* section *Rhynchocystis* appears to have been strongly influenced by climatic oscillations in different ways:

2.1.1 Biogeographic history in the Western Palearctic: The evolutionary history of section *Rhynchocystis* in the Western Palearctic has been mainly shaped by the climatic changes happened during the Mio-Pliocene. It contrast with the primary role attributed to the Pleistocene glaciations in the biogeographic shaping of other plant groups . The progressive cooling and aridification of the Western Palearctic seems to have promoted the retreat of species of *Carex* section *Rhynchocystis* (or of the ancestors that gave rise to these species) to climatically suitable refugial areas. These refuges could have been the Tyrrhenian islands for *C. microcarpa*, SW Asia for *C. agastachys*, and possibly the Iberian Peninsula and / or NW Africa for *C. pendula*. This pattern of distribution leads us to propose a general scenario of isolation in climatic refuges and diversification in the regions located at both ends of the Mediterranean basin during Mio-Pliocene (fig 2. chapter 2) and not only in the Eastern Mediterranean as previously reported for other groups of plants [26]. For the particular case of the couple *C. pendula* and *C. agastachys* the Pleistocene glaciations might also have played a role. The two species seem to have eventually recolonized Europe from their isolated refuges after then glaciations, eventually overlapping in central Europe (Chapter 2-Fig. 1 and Gebauer, personal communication). It appears that they could have undergone ecological differentiation in concert with the genetic and morphological divergence. Thus, while *Carex pendula* is exclusively semi-aquatic, *Carex agastachys* seems to be

more mesic. In *Carex* we find similar cases. For example even among subsp as in *Carex reuteriana*. This species includes two subsp, subsp *mauritanica* and subsp *reuteriana*, being the first strongly Mediterranean, whereas the second requires Atlantic –cooler and wetter- climate ([27] and C. Benítez-Benítez, personal communication). Further studies will be necessary to characterize the contact area of both plants, including ecological observations and the evaluation of hybridization through the test of the molecular signature of the plants where the two species co-occur.

2.1.2 Colonization of Sub-Saharan Africa: Ancestral area reconstruction indicates that *Carex* section *Rhynchocystis* reached the high mountains in tropical and southern Africa, and Madagascar probably from Palearctic ancestors (Fig. 3 of Chapter 2). This supports the conclusions drawn by FULANO & MENGANO [28]. They concluded that the African pan-temperate element is probably entirely of Holarctic origin, which would be the most important source of lineage recruitment of the African high mountain flora of Tropical and Southern Africa. Many of these mountains are separated by vast areas of lower altitude habitat unsuitable for plants adapted to high-alpine conditions, creating a landscape of ‘‘sky-islands’’ [29]. The progressive climatic dessication during the Cenozoic, and the glacial cycles of warming and cooling during the Pleistocene, are probably the reasons for the geographic isolation of plant populations in the high mountainous areas scattered in tropical and southern Africa, as a result of altitudinal migrations [30]. This would have implied allopatric reproductive isolation, determinant for the differentiation processes involved in the origin of *C. penduliformis* and *C. bequaertii*, and in a more incipient stage for the two subspecies of *C. bequaertii* (*subsp. bequaertii* and *subsp. mosii*) (chapter 2).

2.1.3 Colonization of Macaronesia (Azores and Madeira archipelagos):

Macaronesia is considered a "sub-hotspot" of plant biodiversity and endemism in the hotspot of the Mediterranean basin [31]. Vascular plants include about 1,000 taxa in the Azores [32] and 1,240 taxa in Madeira and Selvagens archipelagos (Portal da Biodiversidade dos Açores; http://www.azoresbioportal.angra.uac.pt), considering endemic, native and introduced species and subspecies. Among these, there are about 74 endemic taxa of the Azores [33] and 154 endemic taxa from Madeira [33,34]. The genus *Carex* is represented in the Azores archipelago by 20 species, of which five taxa are considered endemic: *C. hochstetteriana* J. Gay ex Seub. *C. pilulifera* L. ssp. *azorica* (J.Gay) Franco & Rocha Afonso, *C. vulcani* Hochst (Portal da Biodiversidade dos Açores; http://www.azoresbioportal.angra.uac.pt/; [33]) and *C: leviosa* sp.nov. (chapter XX). In Madeira *Carex* is represented by 11 species, with only two taxa endemic, *C. lowei* Bech [34] and *C. pendula* ssp. *myosuroides* comb.nov. It is noteworthy that although in general the number of plant endemics in the Azores is lower than in Madeira [35], the opposite happens in the genus *Carex*, not only with more species but also a higher number of endemic taxa in the Azores than in Madeira.

The volcanic origin of Madeira (<5.6 Ma ago) and Azores (8 Ma ago)[36] implies that the colonization of these islands by native flora has happened through long distance dispersal. At least two independent long-distance dispersal events may have been involved in the colonization of the Azores and Madeira by *Carex* section *Rhynchocystis*. This pattern of recurrent colonization by plants without specialized long-distance dispersal syndromes has been rarely detected in Macaronesia [37]. However these type of plants have been widely successful in the colonization of other oceanic archipelagos as the Galapagos [38]. The dispersal event that allowed *Rhynchocystis* to colonize Azores could have occurred from Europe or northwestern Africa, as can be seen from

the sister relation of *C. leviosa* and *C. pendula*, and the possible origin of this latter in the westernmost Mediterranean (chapter 5). The low molecular differentiation of *C. pendula* subsp. *myosuroide*s with respect to *C. pendula* ssp. *pendula* (see Fig. 3 in Chapter 2 and Fig. 2 in chapter 5), could indicate that the closer situation of Madeira to the Eurasian continent may have allowed the maintenance of a certain gene flow preventing total speciation. Alternatively, it may be that not enough time have passed since the colonization of Madeira to complete the speciation process. Another possible cause of the faster divergence of Azores populations is that if the effective size of the colonizing population was small, the genetic drift could have acted as an important evolutionary force in the absence of gene flow with continental populations, producing a rapid speciation. (REFS.!!!!).

Colonization Inter-Insular in azores

The new species *C. leviosa* appears in all islands of Azorean archipelago with no apparent morphological and genetic relevant variation (see chapter 5). The lack of radiation and the widespread distribution of endemic species in the Azores (known as Azores diversity enigma [35]), have been associated with the combination of several factors: the Azores are young, small and present climatic, edaphic and topographic homogeneity compared to the other Macaronesian archipelagos [39]. This suggests that *C. leviosa* may be an interesting species to carry out future studies on its phylogeography, in order to elucidate how the inter-island colonization of this archipelago took place and as a model to further explore the causes behind the “Azores diversity enigma”.

2.2 Long distance dispersal vs. vicariance in the Rand flora pattern

Rand flora is a biogeographic pattern characterized by a disjunct distribution of related plant lineages in floristic regions around the African continent and separated by hostile climatic inland areas (Le Brun 1971; Bramwell 1985; Andrus et al. 2004; Sanmartín et al. 2010). The current distribution of *Carex* section *Rhynchocystis* (Chapter 2, Fig. 1) conforms to this interesting pattern. Both long distance dispersal and vicariance hypotheses have been postulated to explain Rand flora pattern:

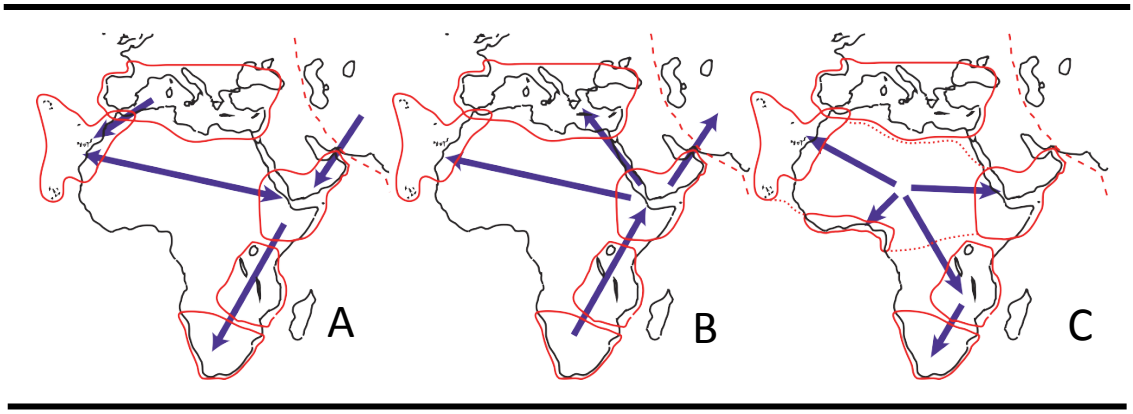


Figure 5. The main hypotheses to explain the origin of the 'Rand Flora' pattern (A) Dispersal southwards to E-S Africa — this pattern is a consequence of immigrants from the Mediterranean region or from west-Asia via the Arabian Plate. (B) Dispersal northwards—the elements of the flora dispersed from south Africa to the Horn of Africa region, and from there northwards and westwards to the SW Paleartic. (C) Vicariance—climatological changes lead to the range fragmentation of a once continuous Miocene (23 Ma) flora. Figure modified from [40,41].

2.2.1 The dispersal hypothesis. The observed disjunctions could be the result of long-distance dispersal events between geographically isolated areas, followed by an in situ diversification of taxa [40]. Despite the fact that no specialized syndrome is known in section *Rhynchocystis* for this type of dispersal, the wide distribution areas of the section suggest effectiveness in the dispersal and colonization of its taxa. Numerous

studies on plant biogeography, especially those using divergence-time estimation, have pointed to the importance of long-distance dispersal (LDD) to explain disjunct distributions, even in organisms with low dispersal capacity [37,42,43]. Several migration routes have been proposed for the Rand flora pattern: To the south, from the Mediterranean region or from southwest Asia (Fig. 5-A), and to the north, from southern Africa, via East Africa, (Fig.5-B) [44].

Our results support several long-distance colonization events in independent lineages of section *Rhynchocystis.* Ancestral range reconstruction analyses and estimations of diversification times indicate that long-distance dispersal events from Eurasia may have been the origin of the African species (*C. bequaertii* and *C. penduliformis*), in congruence with the southwards dispersal hypothesis by [REF; Fig. 5A]. Other disjunctions related to North to South Hemisphere LDD events in *Carex* have been in relation to the origin of the South African endemics *C. ecklonii* Nees, *C. burchelliana* Boeck. (sect. *Spirostachyae*; Escudero et al., 2009; Martín-Bravo & Escudero, 2012) and *C. monotropa* Nees (sect. *Ceratocystis*) [25]. It could have happened also in the related section *Sylvaticae* that shows a similar pattern of disjunction, with species distributed in Eurasia-North Africa (e.g. *Carex hypaneura* V.I. Krecz., *Carex sylvatica* Huds., *Carex cretica* Gradst. & J. Kern.) and in South Africa (*C. rainbowii autores*) [3]. This North to South Hemisphere LDD migration from the Paleartic to sub-saharan Africa has also been documented in other plant species [46–48].

2.2.2 The vicariance hypothesis: The climate changes during late Cenozoic could be the among the main causes that shaped the Rand Flora pattern. The aridification suffered by the African continent produced the fragmentation of flora previously widespread throughout Africa. Due to the strong climatic changes only a few

relict taxa remained and diversified in the continental margins of east and west Africa where the climate was milder ([53;] Fig. 1-C). This hypothesis has been proposed for several groups of plants [30,47,50]. However *Carex* section *Rhynchocystis* seems to have arrived to Africa most probably through long distance dispersal. In the scenario of a vicariance pattern, those Palearctic populations most close to those in Africa (i.e., those from southewestern Asia) would be expected to be the sister group of the latter. However, our study reveals that this is not the case in our group. Indeed, in the moment the vicariant disjunction should have happened (FECHA), another species of the group (*C. agastchys*) was already present in southwestern Asia.

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CONCLUSIONS

1. *Carex* section *Rhynchocystis* is constituted by a monophyletic clade of 6 currently recognised species. Species within section *Rhynchocystis* are monophyletics.

2. *Carex* section *Rhynchocystis* is placed within *Core Carex* together with sections *Sylvaticae*, *Ceratocystis*, *Spirostachyae* and *Rostrales*. The relationships between sections vary depending on the DNA markers used.

3. The origin of Section *Rhynchocystis* is estimated to be 23.07 Ma (28.6–18.36 Ma at 95% HPD), falling in the early Miocene in southwestern Europe–northwestern Africa.

4. The current distribution of *Carex* section *Rhynchocystis* has been strongly influenced by Cenozoic (66.0–2.58 Ma) climatic oscillations, being probably one of the main evolutionary forces.

5. Species of the section *Rhynchocystis* in the Western Palaearctic are Miocene relicts. Putative Miocene-Pliocene refugia were probably located in the Mediterranean peninsulas, as well as in the eastern shores of the ancient Paratethys Sea.

6. *Carex* section *Rhynchocystis* reached, high mountains in tropical and southern Africa, Madagascar and the Macaronesian islands through different colonizations events derived from palearctic ancestors.

7. The integration of molecular and morphological data has contributed to re-evaluate taxonomic relationships of *Carex* section *Rhynchocystis*.

8. *C. bequaertii* and *C. mossii*, are considered as a single species with two subspecies disjunctly distributed: *C. bequaertii* subsp. *bequaertii* and *C. bequaertii* subsp. *mossii.*

9. The subspecies *bequaertii* and *mosii* may have arisen due to a process of vicariance induced by the climatic drying and cooling during the Cenozoic. This supports allopatric speciation as one of the main factors of diversification.

10. *Carex agastachys* L.f. is a cryptic species which had been hidden under the synonymy of its sister species *C. pendula* s.s. during more than 2 centuries.

11. At least two independent events of long distand dispersal could have been involved in the Macaronesian Islands colonization.

12. The Azorean and Madeiran plants are different biological entities. The recently described *Carex leviosa from* Azores archipelago was included within *C. pendula*. It is a genetically and morfologically well defined species that deserves taxonomic recognition while populations from Madeira are an extreme of variation within *C. pendula* with geographical separation that we consider as *C. pendula* subsp. *myosuroides*.

13. Allopatric speciation seems to be the most important mechanism of diversification in *Rhynchocystis*.

Appendices

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Molecular and morphological evidence for a new species from South Africa: *Carex rainbowii* (Cyperaceae)

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ABSTRACT

Carex rainbowii (Cariceae, Cyperaceae), a new species from the Drakensberg mountains (KwaZulu-Natal province), is described and illustrated. It was found in the shady understory of the Afromontane forest in the Cathedral Peak area. An additional, nearby population was also identified based on previously collected herbarium material. Morphological and molecular (cpDNA 5′*trn*K intron and nrDNA ITS and ETS sequences) data were used to evaluate the taxonomic status of these populations and shed light on their systematic placement. Our data strongly support their taxonomic identity and inclusion in *Carex* sect. *Sylvaticae*. The new species can be readily distinguished from other related taxa mainly by the frequently androgynecandrous terminal spike, dense female spikes, hyaline glumes, as well as by some quantitative features. This finding implies a considerable biogeographic disjunction from the mainly Eurasian-North African range of the remaining species of sect. *Sylvaticae*, a pattern also found in the related sections *Ceratocystis*, *Rhynchocystis* and *Spirostachyae*. Comments are provided on previous misidentifications of *C. rainbowii* as the closely related *Carex sylvatica*. Data pertinent to the conservation status of the species are provided.

1. Introduction

With over 2000 species (Reznicek, 1990), *Carex* L. (Cyperaceae, Cariceae) is the most diverse angiosperm genus of Earth's temperate zone (Escudero et al., 2012). It displays the highest species diversity in cold and temperate areas of the Northern Hemisphere, with 527 species recorded in China, 480 in North America (Brach and Song, 2006) and 231 species in Europe and the Mediterranean region (Jiménez-Mejías and Luceño, 2011). Despite the genus being more diverse in northern temperate areas, at least 81 species have been reported from Sub-Saharan Africa and Madagascar (Gehrke, 2011). Among the four traditionally recognized subgenera within *Carex* (*Carex*, *Psyllophora* (Degl.) Peterm., *Vignea* (P. Beauv. ex T. Lestib.) Peterm. and *Vigneastra* (Tuck.) Kük.; Kükenthal, 1909; Egorova, 1999), subgenus *Carex* (c. 1400 species) is the largest and also the most species rich in Sub-Saharan Africa and Madagascar, with 34 species recognized to date (Gehrke, 2011). Eighteen species from this subgenus are currently known in South Africa, 11 of which grow in KwaZulu-Natal (Govaerts et al., 2012).

Within subgenus *Carex*, section *Sylvaticae* Rouy has been frequently subsumed within a widely-circumscribed section *Hymenochlaenae* (Drejer) L.H. Bailey (Kükenthal, 1909). Despite section *Hymenochlaenae* being split up into several sections by Mackenzie (1935), many authors have followed Kükenthal's criteria until recent times (e.g. Reznicek, 1986; Waterway, 1990). Egorova (1999) circumscribed section *Sylvaticae* and considered that some American species from *Hymenochlaenae*, like *Carex debilis* Michx., could be transferred to *Sylvaticae*. Subsequently, the first ITS phylogeny by Hendrichs et al. (2004; later confirmed by Waterway and Starr (2007) and Waterway et al. (2009) revealed the high polyphyly of section *Hymenochlaenae*. Specifically, *Carex sylvatica* Huds. was allied to sections *Rhynchocystis* Dumort., *Ceratocystis* Dumort. and *Spirostachyae* Drejer ex L.H. Bailey, whereas representatives of section *Hymenochlaenae* were distantly related. Under Egorova's (1999) circumscription, section *Sylvaticae* would comprise eight species distributed through Eurasia and North Africa, namely: *C. sylvatica* subsp. *sylvatica* (Europe to Iran and NW Africa) and subsp. *paui* (Sennen) A. Bolòs & O. Bolòs (W Mediterranean), *Carex algeriensis* Nelmes (Algeria), *Carex arnellii* Christ. (Russia, from Europe to Far East), *Carex bostrychostigma* Maxim. (E Asia), *Carex hondoensis* Ohwi (Japan), *Carex hypaneura* V.I. Krecz. (Transcaucasus), *Carex latifrons* V.I. Krecz. (Anatolia to W Caucasus) and *Carex strigosa* Huds. (Europe to N Iran). Subsequently, *C. latifrons* and *C. algeriensis* were included within *C. sylvatica* (= *C. sylvatica* subsp. *latifrons* (V.I. Krecz.) Ö. Nilsson and *C. sylvatica* subsp. *paui* (Sennen) A. Bolòs & O. Bolòs, respectively; see Jiménez-Mejías and Luceño, 2011).

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Finally, *Carex cretica* Gradst. & J. Kern, an endemic to Crete, was recently added to the section (Escudero and Luceño, 2009).

Taxonomy of Cyperaceae in South Africa has remained an active field of research during the last years, with several new species and even a genus very recently described (i.e. Muasya et al., 2011, 2012a, b), although most of them belong to tribe Cypereae, which is more diversified than Cariceae in this region, and includes some species-rich genera like *Cyperus* L. and *Ficinia* Schrad. In a recent field campaign in the Drakensberg mountains (November 2011), we found a population of *Carex* subgenus *Carex* whose morphology was apparently similar to that displayed by some members of *Carex* sect. *Spirostachyae* (especially the Tropical African *Carex fischeri* K. Schum.), a group with four known species in South Africa (*Carex aethiopica* Schkuhr, *Carex clavata* Thunb., *Carex ecklonii* Nees and *Carex burchelliana* Boeckeler; Escudero and Luceño, 2009). However, a closer examination revealed the absence of red crystalline bodies in the utricle epidermis and antiligule, and a smooth utricle beak, indicating a closer relationship to section *Sylvaticae* than to *Spirostachyae*. The presence of section *Sylvaticae* in South Africa was previously noted by Gehrke (2011) based on a specimen collected in KwaZulu-Natal in 1987 by C. Reid (n. 1370; PRE 762278) ('Estcourt district', Ntabamhlope [uThukela District]) and identified as *C. sylvatica*. Another specimen collected in the same place in 1944 ('Weenen district', Ntabamhlope [uThukela district]) by J.P.H. Acocks (n. 10784; PRE 109769; available at JSTOR) was also identified as *C. sylvatica* by C. Archer. An additional population from Eastern Cape (Hogsback) has been reported by C. Archer (pers. comm.). This species has been considered as probably introduced in South Africa (SANBI, 2012; C. Archer, pers. obs.).

The aim of this work is to perform a detailed morphological and molecular study of these taxonomically problematic individuals to evaluate their taxonomic placement and relationships within *Carex*.

2. Material and methods

2.1. Morphological study

In our morphological study, we considered 24 quantitative and 23 qualitative characters, based on the diagnostic characters for the taxonomy of section *Sylvaticae* (Egorova, 1999; Luceño, 2008) and related sections (*Ceratocystis*, Luceño and Jiménez-Mejías, 2008; *Rhynchocystis*, Luceño, 2008; *Spirostachyae*, Escudero and Luceño, 2011). Eleven specimens from the same population (Rainbow Gorge, Cathedral Peak area, KwaZulu-Natal, South Africa; see 3.3) as well as two previously collected specimens from an additional, nearby population (uThukela district; Acocks n. 10784, PRE 109769, available in JSTOR, http://www.jstor.org/ and Reid n. 1370, PRE 762278) were studied. Measurements were made as in our previous taxonomic studies of *Carex* (i.e. Escudero and Luceño, 2011; Martín-Bravo et al., 2012).

2.2. Molecular study

We tested the phylogenetic position of the problematic South African individuals by including: 1) two samples of the South African problematic population from Cathedral Peak area, 2) two species representing sect. *Sylvaticae*: *C. sylvatica* (two samples each in the nrDNA and the cpDNA phylogeny) and *C. cretica* (two samples in the nrDNA phylogeny and one sample in the cpDNA phylogeny) and 3) sequences from two samples representing two species for each of the related sections (Waterway and Starr, 2007): *Carex flava* L. and *Carex viridula* Michx. (sect. *Ceratocystis*), *Carex distans* L. and *Carex punctata* Gaudin (sect. *Spirostachyae*), and *Carex pendula* Huds. and *Carex bequaertii* De Wild. (sect. *Rhynchocystis*). *Carex rostrata* Stokes (sect. *Vesicariae*) and *Carex melanostachya* M. Bieb. (sect. *Tumidae*) were included as the outgroup for the cpDNA analyses and *Carex michauxiana* Boeckeler (sect. *Rostrales*) and *Carex folliculata* L. (sect. *Rostrales*) for the nrDNA analyses. We sequenced and analysed one cpDNA (5′*trn*K intron) and two nrDNA (ITS and ETS) regions, which have been successfully used in molecular systematic studies of the Cariceae (i.e. Escudero and Luceño, 2009; Waterway and Starr, 2007; Jiménez-Mejías et al., 2012). All sequences were downloaded from GenBank except for those from the problematic population as well as four 5′*trn*K intron (*C. sylvatica*, *C. pendula*, *C. bequaertii*) and one ETS sequences (*C. bequaertii*) which were PCR amplified and sequenced for this study (see Appendix A).

Procedures for DNA extraction, amplification and sequencing followed those in Escudero and Luceño (2009) for ITS and 5′*trn*K intron, and in Waterway and Starr (2007) for ETS. We performed Maximum Parsimony and Bayesian Inference phylogenetic analyses as outlined in Martín-Bravo et al. (2007) and Escudero et al. (2008), respectively, for the ITS, ETS and 5′*trn*K datasets individually. Topologies retrieved from the ITS and ETS matrices were congruent (results not shown) and both matrices were therefore combined and analysed. The simplest models of nucleotide evolution that best fit the data for each studied DNA region were HKY for 5′*trn*K intron, GTR + G for ETS and ITS-1, HKY + G for ITS2, and K80 for 5.8S region. Informative indels were coded as a fifth binary character state and analysed with the F81 model of sequence evolution as specified in MrBayes manual (Ronquist and Huelsenbeck, 2003). We also obtained an additional measurement of statistical branch support by performing a Maximum Parsimony fast bootstrap analysis with 1000 replicates as implemented in PAUP (Swofford, 2002).

3. Results and discussion

3.1. Morphological study

The plants from the two KwaZulu-Natal problematic populations (uThukela district: Cathedral Peak area and Ntabamhlope) were compared against the species of sect. *Sylvaticae* (Egorova, 1999; Luceño, 2008). Several morphological features did not match the morphology of any known species of section *Sylvaticae* (Table 1). In particular, these plants frequently display androgynecandrous upper spikes (a previously unknown feature in the section), dense female spikes, sometimes ramified at the base, and hyaline female glumes, which readily allow their distinction from other species in the section.

3.2. Molecular study

The majority rule consensus trees obtained from the Bayesian Inference (Fig. 1) yielded more resolved, but congruent topologies, with respect to the strict consensus trees retrieved from the Maximum Parsimony analyses (not shown). The sectional phylogenetic relationships depicted by the cpDNA (5′*trn*K intron) and nrDNA (ITS-ETS) sequences analysed were significantly different (Fig. 1A, B). Thus, sections *Sylvaticae* and *Spirostachyae* appear as sister groups in the cpDNA analyses (0.97 PP, 57% BS; Fig. 1A), whereas *Sylvaticae* is supported as sister to *Rhynchocystis* in the nrDNA tree (1.0 PP, 89% BS; Fig. 1B). Therefore, nuclear-plastid sequences were not combined but analysed separately. Both the plastid and nuclear analyses support the inclusion of the studied population from Cathedral Peak area within sect. *Sylvaticae*, represented by two samples of *C. sylvatica* and one sample of *C. cretica* in the cpDNA tree (Fig. 1A) and by two samples each of *C. sylvatica* and *C. cretica* in the nrDNA tree (Fig. 1B). Interestingly, the South African population appears as sister to the European-Northern African *C. sylvatica*–*C.cretica* clade in the nrDNA tree (1.0 PP, 98% BS; Fig. 1B), while unresolved in the plastid tree (Fig. 1A). The lower resolution and support for the monophyly of sect. *Sylvaticae* in the cpDNA tree (0.91 PP, 56% BS) than in the nrDNA tree (1.0 PP, 98% BS) are due to the lower number of informative characters in the plastid than in the nuclear matrix (12 vs. 95, excluding the outgroup and the coded indels). Overall, these molecular results support the morphological findings and suggest the

Table 1
Main distinctive characters between *C. rainbowii* and Euro-North African species of section *Sylvaticae*.

	C. rainbowii	*C. sylvatica* subsp. *sylvatica*	*C. sylvatica* subsp. *paui*	*C. cretica*	*C. strigosa*
Distribution	South Africa	Europe, scattered in SW Asia and NW Africa	NE Spain, NW Africa	Crete	S, C, W Europe, SW Asia (Caucausus, N Iran)
Rhizome	Caespitose	Caespitose	Caespitose	Caespitose	Creeping
Longest stem (mm)	710	1000	2000	450	1000
Leaf upper side	Rough just in the upper 1/3	Smooth to slightly rough	Strongly rough	Smooth	Smooth
Leaf width (mm)	(4.7)6.0–10.0(11.1)	(2)4–7(8)	(6)8–12	(1.5)2.0–2.5(3.0)	(2.9)5.0–6.5
Upper spike	Male or, more frequently, androgynecandrous	Male	Male	Male	Male
Male spikes number	0–1(2)	1(2)	(1)2–4(7)	1	1
Female spike	Up to 51 mm long, sometimes ramified at the base, dense	Up to 45 mm long, simple, lax	Up to 75 mm long, sometimes ramified at the base, lax	Up to 15(20) mm long, simple, lax	Up to 60 mm long, simple, lax
Female glume	Hyaline	Pale brown with wide hyaline margin	Pale brown with wide scarious margin	Hyaline to pale brown	Hyaline, pale brown or purplish
Utricle length (mm)	(3.0)4.0–4.5	(3.8)4.0–5.0	(4.0)4.5–5.3	2.5–2.9(3.2)	(2.7)3.0–3.5(4.0)
Utricle beak	Smooth	Smooth	Sometimes aculeolate	Smooth	Smooth

taxonomic identity of the studied South African populations and their sectional circumscription within sect. *Sylvaticae*.

3.3. Description of new species

Although apparently close to *C. sylvatica*, *C. rainbowii* is not morphologically more similar to *C. sylvatica* than to other species from sect. *Sylvaticae* (Table 1). In addition, phylogenetic trees (Fig. 1) do not clearly indicate that *C. rainbowii* is closer to *C. sylvatica* than to the other sampled *Sylvaticae* species (in fact, in the nrDNA tree *C. sylvatica* is closer to *C. cretica* – although with low support – than to *C. rainbowii*; Fig. 1B). In our opinion, despite the small amount of studied material, the congruent and distinct set of clear-cut morphological characters (Table 1), together with the evidence from the molecular phylogenetic analyses (Fig. 1), warrants formal taxonomic recognition at the species level, which leads us to propose the following new species.

3.3.1. *C. rainbowii Luceño, Jim. Mejías, M. Escudero & Martín-Bravo, sp. nov.* (Fig. 2)

Similar to *C. sylvatica*, from which it differs mainly by its frequently androgynecandrous upper spike, the dense female spikes and the hyaline female glumes.

Type: South Africa. KwaZulu-Natal Province, uThukela district (2829): Cathedral Peak Area, Rainbow Gorge, shady understory of montane forest, 1525 m, 13 Nov 2011, S. Martín-Bravo 120SMB11 & M. Luceño (PRE, holo.; BOL, BM, K, M, MA, NU, UPOS, iso.).

Etymology — this new taxon is named after the Rainbow Gorge, the place in the Drakensberg Mountains (KwaZulu-Natal, South Africa) where the species was found (there are numerous waterfalls in this gorge producing rainbows). In addition, the species is endemic to South Africa, which is popularly known as the Rainbow Nation. Following the International Code of Botanical Nomenclature (ICBN, Rec. 60D; McNeill et al., 2006) for geographical names, the specific epithet should be "*rainbowensis*", but we have used "*rainbowii*", since the rainbow symbolizes the peace and the freedom. To both qualities this species is also dedicated.

Plant caespitose. Stems 450–710 mm, sharply trigonous above, smooth. Leaves slightly shorter, as long as or longer than stems, (4.7) 6.0–10.0(11.1) mm wide, plicate, soft, scabrid on the edges, except basal parts, and on both faces in the apical part; ligule 1–3(8) mm, apex usually rounded, rarely subacute; antiligule absent; lowermost sheaths of the flowering stems foliose, the 2–3 lowermost of the sterile shoots scale-like, straw-coloured to light brown, entire to slightly fibrose. Inflorescence 180–350 mm. Lowest bract slightly longer or shorter than inflorescence; sheath 22–55 mm long, the inner side green. Spikes 5–6, heteromorphic, with 1 apical male or androgynecandrous, sometimes with a small male or androgynous spike at its base, and 4–5 lateral female. Apical spike 30–45 mm × 1.8–8.7 mm, fusiform. Female spikes 28–51 × 5–8 mm, arising singly, dense-flowered except sometimes in basal parts, the lowest with a peduncle up to 185 mm, the 1–3 lowest pendulous. Male glumes 5.0–6.2 × 1.0–1.8 mm, lanceolate, narrowly ovate or oblong-elliptic, hyaline to straw-coloured, with a narrow green midrib, uninervate, acuminate to aristate. Female glumes (3.0) 3.5–4.0(5.0) × (1.1)1.3–1.8 mm, ovate, hyaline, with a narrow green midrib, 1–3 nervate, aristate, with an arista up to 1.5(1.8) mm, sometimes reduced to a short mucro. Utricles (3.0)4.0–4.5 × (1.0)1.2–1.6 mm, ovate to ellipsoid-trigonous, straight, with 2 well marked nerves, sometimes with some additional faint nerves, abruptly narrowed into a beak, greenish-brown; beak 1.2–2.0 mm, slightly bidentate to nearly truncate, with a deeper dorsal sinus, smooth. Achenes 2.0–2.8 × 1.1–1.3 mm, elliptic, trigonous.

3.3.1.1. Distribution (Fig. 3). South Africa, KwaZulu-Natal (NAT; Brummit, 2001), known only from two collecting sites in the Drakensberg Mountains (uThukela district): Cathedral Peak area and Ntabamhlope.

3.3.1.2. Ecology. In the holotype population, plants were growing in the shady understory of a montane forest dominated by *Podocarpus latifolia* (Thunb.) Mirb. and *Carissa bispinosa* Desf. Other accompanying observed species were *Celtis africana* Burm. f., *Carex spicato-paniculata* C. B. Clarke, *Schoenoxiphium lehmannii* (Nees) Steud., *Dietes iridioides* (L.) Klatt and *Blechnum giganteum* Schltdl. The habitat in the paratype population appears to be similar, with plants found in damp and shady places in a forest. c. 1500–1700 m.

3.3.2. Phenology. (Jul-)Aug–Jan(-Feb)

3.3.2.1. Conservation. Because the species is currently known from only two collecting sites in South Africa, we hereby summarize the available information for assessing the conservation status of *C. rainbowii*, based on IUCN Red List categories, criteria, and guidelines (IUCN, 2001, 2011). The known collecting sites would correspond to only two subpopulations (IUCN, 2011) in an extremely reduced area of occupancy (AOO) of 8 km^2 (grid size 4 km^2). We could not precise the exact number of locations (1–2) due to no information available about threatens affecting subpopulations (IUCN, 2001). However, this geographic range and number of locations (maximum two) would classify the species as "Endangered" under criteria B2, if appropriate knowledge of subcriteria b and c were available. Unfortunately, no information is available for these subcriteria, which refer to a verifiable continuing decline (subcriterion b) or extreme fluctuation (subcriterion c) in any of the extent of occurrence, AOO, area, extent, and/or quality of the habitat; number of locations; and number of mature individuals (IUCN, 2001, 2011). Alternatively, it could be classified as "Vulnerable" under

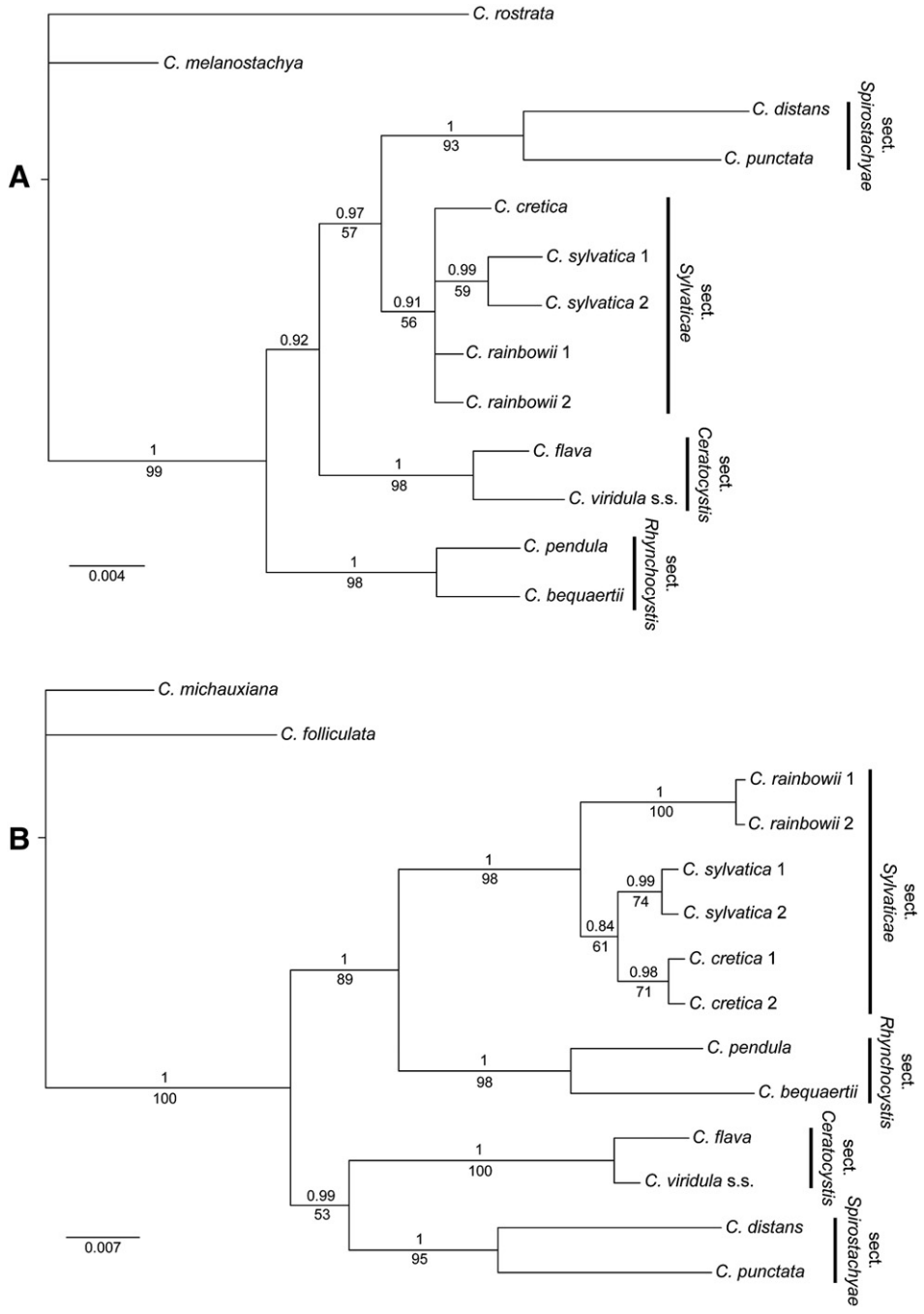

Fig. 1. Majority rule consensus tree of the 37,500 trees retained in the Bayesian inference analyses including two samples of *Carex rainbowii* plus representatives of sections *Sylvaticae* (*C. cretica*, *C. sylvatica*), *Spirostachyeae* (*C. distans*, *C. punctata*), *Ceratocystis* (*C. flava*, *C. viridula*) and *Rhynchocystis* (*C. pendula*, *C. bequaertii*) and two species used as the outgroup for the phylogenetic analyses. Informative indels were coded and analysed as specified in the text (see 2.2). Posterior probabilities and bootstrap values are given above and below branches, respectively. (A) cpDNA tree (5′trnK intron); (B) nrDNA tree (ITS-ETS combined).

criterion D2, because the AOO and the number of locations are smaller than 20 km^2 and fewer than five, respectively. However, this criterion requires that a plausible future threat for the species survival is identified. However, because the species can be locally frequent (ca. 100 plants seen in the holotype population, Martín-Bravo and Luceño, pers.obs.; "frequent" and "locally fairly common" in the paratype population, Acocks and Reid, *in sched.*, respectively), and at least the Rainbow gorge population is situated on protected land (Ukhahlamba Drakensberg park), no clear future threat is currently apparent as required by criterion D2. To sum up, the necessary information for a complete conservation assessment was insufficient to meet some of the criteria and subcriteria required by the IUCN to qualify the species under the different threatened categories (Critically Endangered, Endangered, Vulnerable). Therefore, the species should be classified as "Data Deficient" at the present time, although it is likely that it would deserve protection under the IUCN guidelines if more information was obtained. An additional population of this species could be present in the Eastern Cape province (Hogsback; C. Archer, pers. comm.), which would considerably increase the extent of occurrence (EOO) of this species (IUCN, 2001, 2011).

3.3.2.2. Biogeographic observations. The fact that the paratype population (Ntabamhlope) of *C. rainbowii* had been previously misidentified as *C. sylvatica* (Gehrke, 2011), indicates that *C. sylvatica* should be

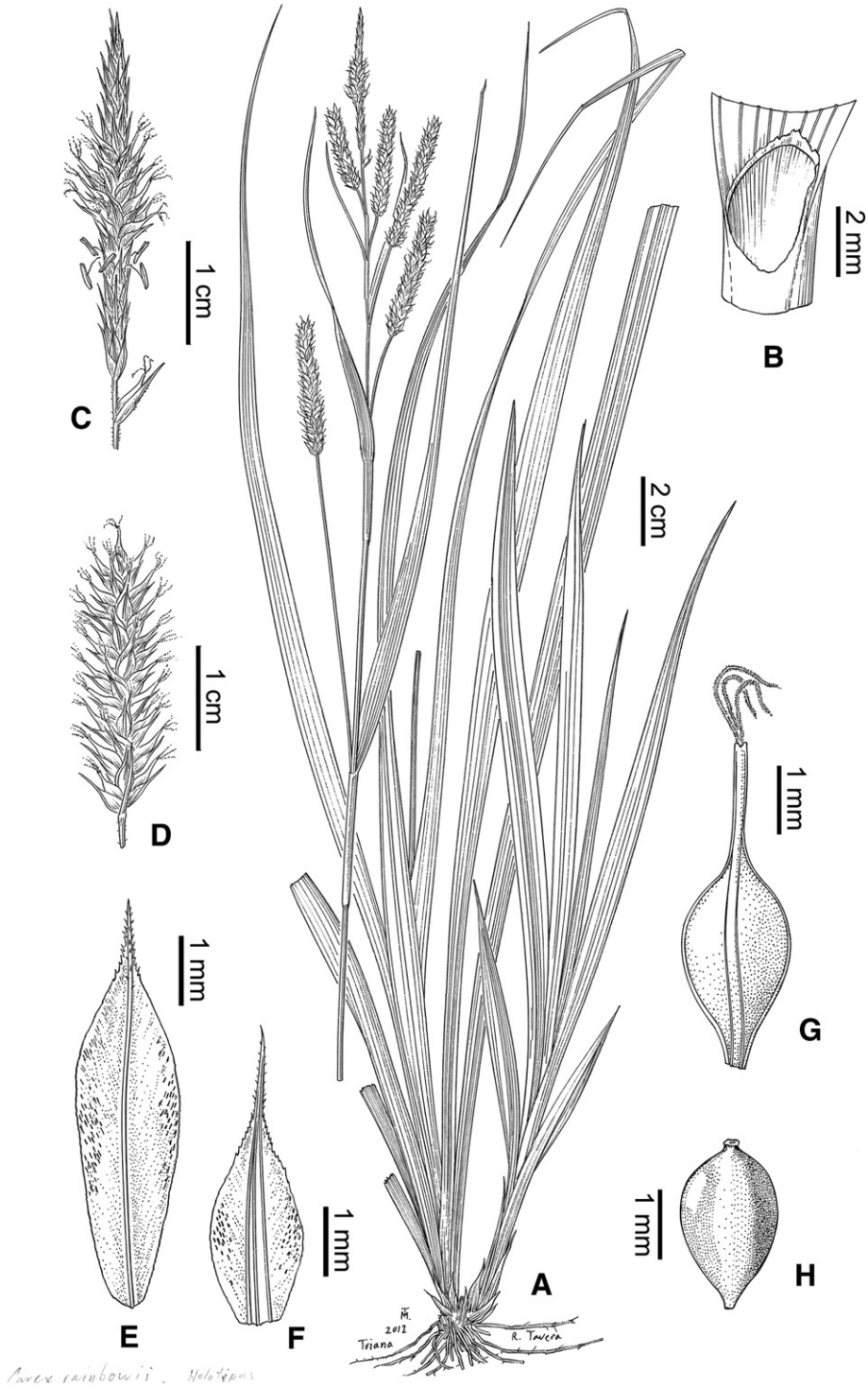

Fig. 2. Analytical drawing of the holotype material of *Carex rainbowii*. A. plant; B. ligule; C. androgynecandrous spike; D. female spike; E. male glume; F. female glume; G. utricle; H. achene (South Africa, KwaZulu-Natal, uThukela district, Cathedral Peak area, Rainbow Gorge, S. Martín-Bravo 120SMB11 & M. Luceño, UPOS). — Drawing by Rodrigo Tavera.

excluded from the checklist of alien flora of South Africa (SANBI, 2012). The presence of this new species in South Africa implies a hitherto unknown disjunction with respect to the mainly Eurasian-North African distribution of the remaining species of section *Sylvaticae*. Interestingly, this biogeographic pattern has also been found in several of the related *Carex* sections. In section *Spirostachyae*, ancestral range reconstruction analyses and estimations of diversification times indicate that long-distance dispersal events from Eurasia may have been the origin of the two South African endemic species (*C. ecklonii* and *C. burchelliana*; Escudero et al., 2009; reviewed in Martín-Bravo and Escudero, 2012). For the origin of the *C. aethiopica–C. clavata* complex, both long-distance dispersal from Eurasia and ecological vicariance with its East Tropical African congeners have been proposed (Gehrke and Linder, 2009; Escudero et al., 2009). In section *Ceratocystis*, the Drakensberg endemic *Carex monotropa* Nees is likely the result of a North to South Hemisphere migration (Jiménez-Mejías et al., 2012). Finally, section

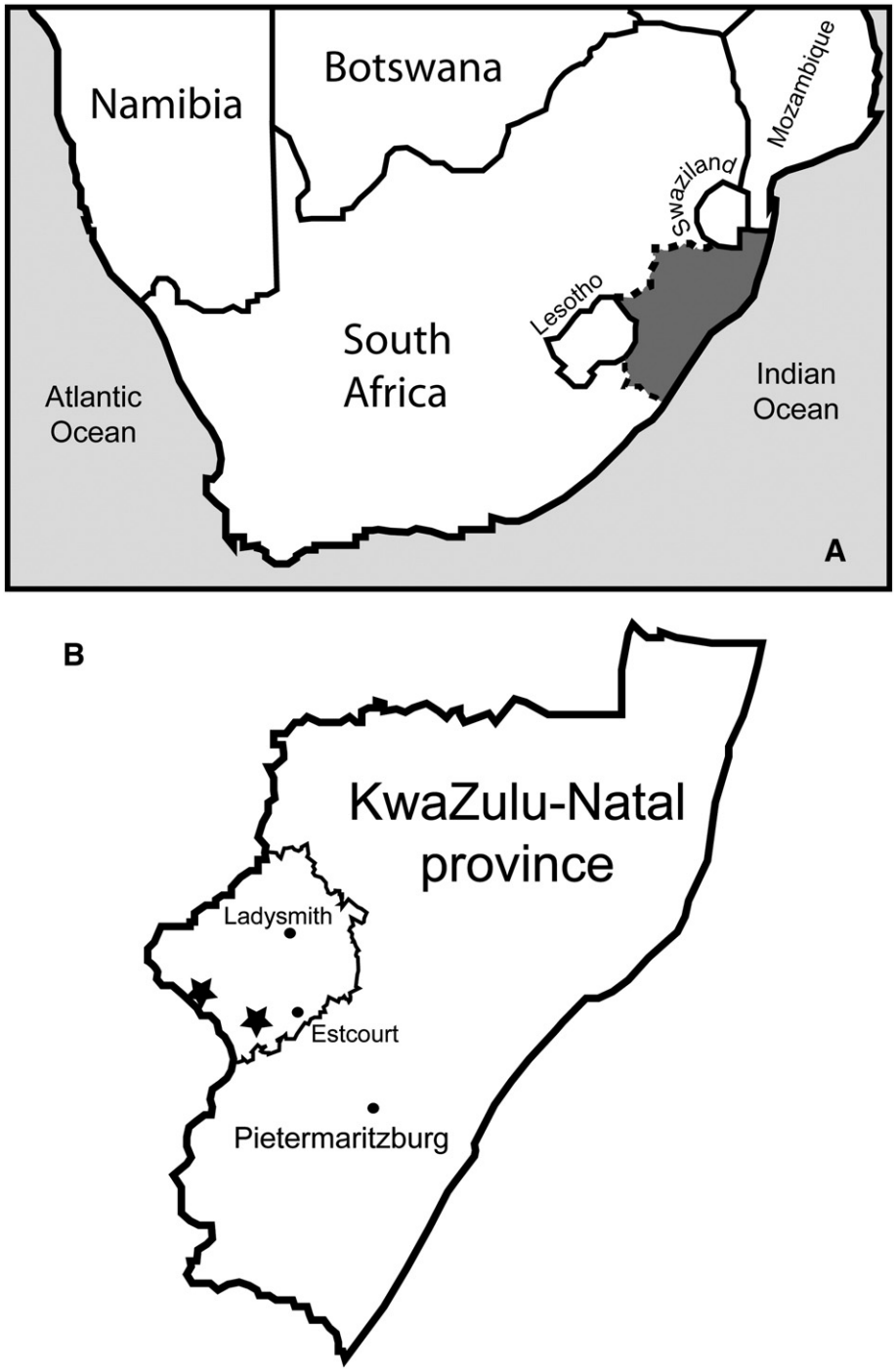

Fig. 3. Distribution of *Carex rainbowii*. A: the shaded area corresponds to KwaZulu-Natal province. B: the two studied populations are depicted by stars within the uThukela district in KwaZulu-Natal province. Dots indicate the capitals of the province and the district and the nearest main city to the populations.

Rhynchocystis also shows a similar pattern of disjunction, with its species displaying a Euro-North African-Western Asian (*C. pendula*, *Carex microcarpa*) or Sub-Saharan (*Carex moosi*, *C. bequaertii*, *C. penduliformis*) range. Comparative biogeographic studies of these groups are needed to uncover if these shared patterns are reflecting common evolutionary histories.

3.3.2.3. Additional specimens studied (paratypes). South Africa: KwaZulu-Natal province, 'Weenen district' [uThukela district] (2929): Ntabamhlope, near police post, damp place in forest, 5500′, 19 Nov 1944, J.P.H. Acocks 10784 (PRE, photo!); Idem, 'Estcourt district' [uThukela district] (2929): near Ntabamhlope, c. 2 km on road to Kamberg from White Mountain Resort, small forest patch, along stream bed in forest, 30 Jan 1987, C. Reid 1370 (PRE, photo!; GENT, J, NH, iso.).

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Appendix A

Sampling and GenBank number accessions for ITS and ETS sequences. *C. bequaertii* De Wild. (EU288576, KC122390*), *C. cretica* Gradst. & J. Kern (DQ384117, -), *C. cretica* (DQ384118, -), *C. distans* L. (DQ384127, -), *C. flava* L. (AY278310, AY757657), *C. folliculata* L. (AY757601, AY757662), *C. michauxiana* Boeckeler (AY757602, AY757663), *C. pendula* Huds. (AY757600, AY757661), *C. punctata* Gaudin (DQ384182, AY757659), *C.rainbowii* Luceño, Jim. Mejías, M. Escudero & Martín-Bravo (KC122380*, KC122388*), *C.rainbowii* (KC122381*, KC122389*), *C. sylvatica* Huds. (AY757599, AY757660), *C. sylvatica* (AY278306, -), *C. viridula* Michx. (JN634666, AY757658). B. Sampling and GenBank number accessions for 5′*trn*K intron sequences. *C. bequaertii* (KC122385*), *C. cretica* (EU812677), *C. distans* (EU812650), *C. flava* (JN627711), *C. melanostachya* M. Bieb. (JN627756), *C. pendula* (KC122384*), *C. punctata* (EU812618) *C. rainbowii* (KC122382*), *C. rainbowii* (KC122383*), *C. sylvatica* (KC122386*), *C. sylvatica* (KC122387*), *C. rostrata* Stokes (EU81268), *C. viridula* (JN627726). Asterisks depict new sequences obtained in this study.

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Molecular and morphological data resurrects the long neglected *Carex laxula* (Cyperaceae) and expands its range in the Western Mediterranean

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Abstract

Carex sylvatica subsp. *paui* (Sennen) A. Bolòs & O. Bolòs is a poorly studied taxon considered endemic from a few places in the western Mediterranean. It has been frequently misidentified as *C. sylvatica* subsp. *sylvatica*. To date, it has been reported only from the NE Iberian Peninsula and NW Africa. We use molecular (nuclear ribosomal –ITS–and plastid–5´trnK– sequences) and morphological data to shed light on the taxonomic circumscription and distribution of this taxon, especially regarding its distinction from the type subspecies. The genetic data support the recognition of *C. sylvatica* subsp. *paui* as an independent species, and confirm new records from the Mediterranean Balearic and Tuscan Archipelagos (Elba Island). It implies a considerable increase in its range and a new species for the Italian flora. Strikingly, the morphometric analyses revealed that the type voucher of *C. laxula* Tineo ex Boott from Sicily corresponds to what has been called *C. sylvatica* subsp. *paui*, thus becoming the prior name at species rank. We also provide notes on the ecology of the taxon.

Keywords: Balearic archipelago, *Carex* sect. *Sylvaticae*, ITS, Mediterranean flora, taxonomy, Tyrrhenian, Tuscan archipelago, 5´trnK

Resumen

Carex sylvatica subsp. *paui* (Sennen) A. Bolòs & O. Bolòs es un taxón poco estudiado considerado endémico de unos pocos lugares del oeste del Mediterráneo. Ha sido frecuentemente confundida con *C. sylvatica* subsp. *sylvatica*. Hasta la fecha, se ha citado solo de Cataluña y el Noroeste de África. Utilizamos datos moleculares (la región ribosómica ITS y la plastídica 5´trnK-) y morfológicos para estudiar la delimitación

taxonómica y distribución de este taxón, especialmente en relación con la subespecie tipo. Los datos genéticos apoyan el reconocimiento de *C. sylvatica* subsp. *paui* como una especie independiente, y confirma su presencia en Baleares y el archipiélago Toscano (isla de Elba). Esto implica un considerable aumento de su distribución y un nuevo taxón para la flora de Italia. Sorprendentemente, los análisis morfométricos han revelado que el espécimen tipo de *C. laxula* Tineo ex Boott de Sicilia se corresponde con la misma entidad que *C. sylvatica* subsp. *paui*, lo que convierte a este nombre como el prioritario a nivel de especie. Proporcionamos además comentarios sobre la ecología del taxon.

Palabras claves: archipiélago Toscano, Baleares, *Carex* sect. *Sylvaticae*, flora Mediterránea, ITS, taxonomía, Tirreno, 5´trnK

1. Introduction

The genus *Carex*. L. with more than 2,000 species comprises about 40% of the total number of family Cyperaceae (Reznicek 1990; Global *Carex* Group, 2015). It has a cosmopolitan distribution, with most species diversity in temperate regions of the Northern Hemisphere. Section *Sylvaticae* Rouy is a morphologically well-defined small section that currently comprises six species (see Table 1) distributed in temperate Europe, Western Asia, North and South Africa. It is placed in subgenus *Carex*, and is included in a well-supported clade together with sections *Rhynchocystis* Dumort., *Ceratocystis* Dumort., *Spirostachyae* Drejer ex Bailey and *Rostrales* Meinsh. (Global *Carex* Group, 2016). *Carex sylvatica* is native to the Old World and it is the most widespread species of the section *Sylvaticae*, being distributed in Europe, Western Asia, and North Africa; it has also been reported as introduced in North America and New Zealand (Govaerts et al., 2016). Three subspecies are currently recognized: subsp. *latifrons*, subsp. *sylvatica* and subsp. *paui* (Egorova, 1999; Jiménez-Mejías & Luceño, 2011). Subspecies *latifrons* is distributed in SW Asia, from Turkey to Western Caucasus, and inhabits forests and wet meadows (Nilsson, 1985). *S*ubspecies *sylvatica* is widely distributed across most Atlantic and Eurosiberian Europe and Western Asia (Egorova, 1999; Jiménez-Mejías & Luceño, 2011). Eventually, the subspecies *paui* has been considered a Mediterranean element with a hitherto known distribution restricted to the NE Iberian Peninsula (Catalonia and Navarra; Bolòs & Vigo, 2001; Luceño & al., 2008; Aizpuru & al., 1999, Jiménez-Mejías & Luceño, 2011) and NW Africa (Algeria

and Tunisia, previously regarded as *C. algeriensis* Nelmes by Maire, 1957; synonymized to *C. sylvatica* subsp. *paui* (Jiménez-Mejías & Luceño, 2011; Martín-Bravo & al., 2013).

Despite being an especially well-known area from the floristic point of view, the Mediterranean Basin is still revealing some taxonomic and chorological novelties. Such new findings have been especially remarkable in family Cyperaceae due to its complicate taxonomy. Recent studies have revealed the previously unknown presence of several species in different areas: *Schoenoplectus corymbosus* (Roth ex Roem. & Schult) J. Raynal (Jiménez-Mejías & al., 2007), *Cyperus glaber* L. (Verloove & Mesterházy, 2013), both unknown until then to Spain; *Cyperus erythrorrhizos* Muhl. (Verloove & Saiani, 2015) newly reported for Europe as introduced in Italy); and even the description of new species (*Carex castroviejoi* Luceño & Jim.Mejías, Jiménez-Mejías & Luceño, 2009; from Greece).

The finding of *Carex* sect. *Sylvaticae* materials resembling *C. sylvatica* subsp. *paui* from the Balearic and Tuscan Archipelagos and Sicily led us to perform a revision of the taxonomy of *C. sylvatica*-like plants in the Western Mediterranean Basin. Interestingly, the voucher from Sicily is the type material of *C. laxula* Tineo ex Boott, which has been to date considered a synonym of *C. sylvatica* subsp. *sylvatica* (Govaerts & al., 2016).

In this paper we use sequences from two genomes (nrDNA ITS and ptDNA 5' trnK) and morphological data from herbarium specimens to clarify the taxonomic identity of these problematic populations. These molecular regions have been widely and successfully used for systematic purposes in *Carex*, including section *Sylvaticae* (Martín-Bravo & al., 2013) and other closely related groups (i.e. Escudero & Luceño, 2009; Jiménez-Mejías & al., 2012). Interestingly, to the best of our knowledge, *C. sylvatica* subsp. *paui* has never been included in a molecular phylogenetic study. We aim to gain insights on the delimitation and distribution of *C. sylvatica* subsp. *paui*, especially in regards to its distinction from its close relative the subspecies *sylvatica*.

2. Materials and Methods

2.1. Molecular study

We performed a phylogenetic reconstruction to check the phylogenetic placement of the Balearic and Tuscan materials and to assess the degree of genetic differentiation between *C. sylvatica* subsp. *sylvatica* and subsp. *paui*. Our sampling (Appendix 1)

included: 1) 18 samples of *C. sylvatica* subsp. *sylvatica*, selected to representatively cover its distribution area; 2) five samples previously classified as *C. sylvatica* subsp. *paui* from N Africa and N Spain; 3) three samples of the C. *sylvatica* subsp. *paui*-like plants from the Balearic and Tuscan Archipelagos; 4) four samples of two other species of sect. *Sylvaticae* (*C. rainbowii*, and *C. cretica*) and 5) six samples representing two species of each of the three sections phylogenetically closely related to *Sylvaticae* (Waterway & Starr, 2007; Martín-Bravo & al., 2013): *C. demissa* Hornem., and *C. flava* L. (sect. *Ceratocystis*); *C. distans* L. and *C. punctata* Gaudin (sect. *Spirostachyae*); *C. pendula* Huds. and *C. bequaertii* De Willd. (sect. *Rhynchocystis*). DNA was extracted from the specimens using a DNeasy Plant Mini Kit (Qiagen). Materials were PCR-amplified following PCR conditions from Escudero & Luceño (2009). Sequence chromatograms were visualized and edited using the program Geneious v. 6.1.7 (Biomatters). Two matrices were built, one containing only the ITS sequences (nrDNA matrix), and another one with the 5'trnK sequences (ptDNA matrix). Informative indels were coded as a binary character. We performed Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic analyses on each matrix as explained in Escudero & al. (2008), Martín-Bravo & al. (2013), and Villaverde & al. (2015) for the ITS and 5´ trnK datasets individually. ML analyses were run in RAxML v. 7.2.6 (Stamatakis, 2010), with a GTR+GAMMA model of sequence evolution, as implemented in a Phylocluster (California Academy of Sciences). Bootstrap support for branches was calculated with 1000 replicates. BI analyses were run in MrBayes v. 3.2.5 (Ronquist & Huelsenbeck, 2003). Four simultaneous Markov Chain Monte Carlo (MCMC) chains were run for 5 million generations, sampling trees every 100 generations. The simplest models of nucleotide evolution that best fit the data for each studied DNA region were HKY for 5´trnK, HKY+I for ITS1 and ITS2 and JC for 5.8S region. Characters corresponding to coded indels were analysed with a F81 model. Congruence between the two resulting topologies was checked by eye and using Hompart test as implemented in PAUP* v. 4.0b10 (Swofford, 2002) in the same Phylocluster (California Academy of Sciences). As no significant incongruences were found (see results), the two data sets were combined into a total evidence matrix (combined matrix), which was analysed again using ML and BI. Finally, in order to assess and compare the degree of genetic differentiation between the studied taxa of section *Sylvaticae*, we measure the Kimura-2-parameter genetic distance with MEGA ver. 5.2 (Tamura & al., 2011), using the ITS, 5´trnK and concatenated sequences.

2.2 Morphological study

Twenty-two herbarium specimens of typical *C. sylvatica* subsp. *sylvatica* (Appendix 1) and eleven specimens previously classified as subsp. *paui* (Appendix1; including the three vouchers from Balearic and Tuscan Archipelagos, and the type of *C. laxula* from Sicily) were included in our study. For the morphological characterization of the materials we measured the diagnostic characters reported in previous taxonomic studies considering *C. sylvatica* and allies (Chater, 1980; Nilsson, 1985; Luceño & al., 2008; Egorova, 1999; Martín-Bravo & al., 2013), as well as additional characters derived from our observations, making a total of 32 quantitative and one qualitative trait (Table 2). Measurements were taken using a binocular micrometer (Nikon SMZ645), with the exception of the largest characters, which were measured using a standard 30-cm rule.

All statistical analyses were performed using the software SPSS Statistics 20 (IBM Corp., New York, Armonk). First, we chose those variables with a higher correlation level (>0.8). Secondly, we removed those variables that contributed the less to the first significant principal components. As the characters were removed under such premises, a better clear morphological discontinuity between the two subspecies could be achieved evaluating the structure by principal component analysis (PCA). A total of seven variables were kept for the final analysis. We performed boxplots for these variables in order to show the degree of overlapping between the two considered taxa. In the boxplots the Navarran samples previously considered to be subsp. *paui* were considered under *C. sylvatica* subsp. *sylvatica* (see results).

3. Results

3.1.Molecular study

ITS and 5'trnK sequences from the three problematic *Carex sylvatica* subsp. *paui*-like vouchers from the Balearic and Tuscan archipelagos clustered with those from typical specimens from NW Africa and Catalonia, whether analysed separately (nuclear vs. plastid data sets) or combined (Figs. 1, S1 and S2 in supplementary material). The Navarran sample previously classified as subsp. *paui*, however, grouped with *C. sylvatica* subsp. *sylvatica*. The phylogenetic relationships revealed by the separate nrDNA and ptDNA trees and the combined tree were somehow different (Figs. 1, S1 and S2, respectively), but were shown not to be significantly incongruent by the Hompart test ($p>0.05$). The topology of the combined tree mostly agreed with the one

yielded by the nrDNA data. Section *Rhynchocystis* was strongly supported as sister group to section *Sylvaticae* in the combined and nrDNA tree (1.0 PP, 100% BS; 1.0 PP, 98% BS; Fig 1 and S1, respectively), whereas in the ptDNA tree sections *Rhynchocystis* and *Ceratocystis* were resolved as sister groups and in turn sister to section *Sylvaticae*, but without significant support (Fig. S2). ITS and combined analyses yielded a strongly supported monophyletic section *Sylvaticae* (1.0 PP, >90% BS; Figs. 1, S1), whereas ptDNA analysis showed a moderate support for the monophyly of section *Sylvaticae* (0.91 PP, <50% BS, Fig. S2). The South African *C. rainbowii* was resolved as sister to the remainder lineages of the section (*C. sylvatica* and *C. cretica*) in the combined and nrDNA trees (Fig. 1, S1, respectively), with *C. cretica* in turn being resolved as sister to *C. sylvatica* only in the combined analysis (Fig. 1). The three species collapsed in a basal polytomy in the ptDNA tree (Fig. S2). *Carex sylvatica* monophyly showed low support in the combined (PP<0.9, BS 65%; Fig. 1), while its sequences collapsed in a polytomy in the nrDNA and ptDNA phylogeny (Figs. S1, S2). Interestingly, while *C. sylvatica* subsp. *sylvatica* was only recovered as monophyletic in the ptDNA tree (0.99 PP, 81% BS; Fig. S2), subsp. *paui* was strongly supported as a monophyletic group by all markers and analyses (1.0 PP, >90% BS; Figs. 1, S1, S2).

The calculated genetic distances (Table 3) revealed that *C. sylvatica* subsp. *sylvatica* (including the Navarran sample mentioned above) was more distantly related to *C. sylvatica* subp. *paui* (ITS: 0.016; 5´trnK: 0.007; concatenated sequences: 0.009) than to *C. cretica* (ITS: 0.004; 5'trnK: 0.005; concatenated sequences: 0.006) and even to *C. rainbowii* for 5´trnK sequences (0.003).

3.2. Morphological study

For the sake of simplicity, and according to the molecular results, we considered the Navarran samples previously classified as subsp. *paui* to be *C. sylvatica* subsp. *sylvatica*.

The PCA including only seven variables (SL, INFL, UL, UBL, SPKMN, SPKAN and PAP, see Table 2) revealed a clear separation between *C. sylvatica* subsp. *sylvatica* and *C. sylvatica* subsp. *paui* (Fig. 2). The first three principal components (PCs) accounted for 73.51% of the total variance (31.74%, 23.88% and 17.89% respectively). The characters that contributed the most to the first three components were related to plant and utricle sizes, as well as the androgynous spike number (Table S1).

Boxplots showed that at least UL, UBL, SPKAN and PAP displayed less than 25% overlap between the two considered taxa (Fig.3).

Table 4 summarizes those diagnostic characters that distinguish between both subspecies according to our own results and previous studies (Luceño & al., 2008; Martín-Bravo & al., 2013).

4. Discussion

4.1. Carex sylvatica subsp. paui should be considered a distinct species: Carex laxula

Our molecular study revealed that the samples that we considered to be *C. sylvatica* subsp. *paui* formed a well-supported clade, recovered as sister to *C. sylvatica* subsp. *sylvatica* in the combined tree (Fig. 1). Moreover, the genetic distance between these two taxa was larger than between *C. sylvatica* subsp. *sylvatica* and *C. cretica* (Table 3), being this latter taxon usually considered a "good" species, fairly morphologically distinct from any other member of section *Sylvaticae* (Escudero & Luceño, 2009; Martín-Bravo et al., 2013). In addition, the results retrieved by the morphometric study revealed a good degree of differentiation and a number of diagnostic characters between the two taxa despite the small number of specimens considered (Fig. 2), including the type of *C. laxula* in the study. In addition, at least four characters displayed no or few (<25%) overlap (Fig 3, Table 2). The number of male spikes has been considered the most important and clear diagnostic character in previous studies (Table 4). Our study has revealed the existence of other important distinctive characters between both taxa: the number of androgynous spikes, the length of the stem, the inflorescence, the utricle and the beak utricle, all of which are higher in subspecies *paui* than subspecies *sylvatica* (Fig.4). In addition, the adaxial leaf surface is strongly rough in subspecies *paui* whereas smooth or slightly rough in subspecies *sylvatica* (Fig. 4, Table 3). All the evidence presented lead us to consider that *C. sylvatica* subsp. *paui* should be considered at species rank, being its priority name *C. laxula*.

4.2. Carex laxula new for the Balearic and Tuscan archipelagos and excluded from Navarra

Our study clearly shows that the specimens from the Balearic and Tuscan archipelagos, and the type of *C. laxula*, formed indeed a well-defined morphogroup with part of the samples from the Iberian Peninsula (Catalonia) (Fig. 2). This is also in

agreement with the phylogenetic placement of the samples included in the molecular study (Figs. 1, S1 and S2). On the contrary, the Navarran samples previously classified as subsp. *paui* have been retrieved within the variation of *C. sylvatica* subsp. *sylvatica* in both the molecular and the morphometric studies.

Carex sylvatica subsp. *paui* was first described at the specific rank (*C. paui*; Sennen, 1925), but this taxon was not widely accepted until it was proposed at the subspecific rank (*C. sylvatica* ssp. *paui*, Bolòs & Bolòs, 1950) so as recognized by later authors (Luceño & al., 2008; Jiménez-Mejías & Luceño, 2011; Govaerts & al., 2016,). At first, this taxon was only known from Spain (Luceño, 1994; Luceño & al., 2008). Later, the populations of *C. algeriensis* from Algeria and Tunisia (Maire, 1957) were reported to be conspecific with *C. sylvatica* subsp. *paui* on the basis of morphological data (Jiménez-Mejías & Luceño, 2011; Martín-Bravo & al., 2013). Such consideration is also confirmed by the results of our phylogenetic study (see the sample from Tunisia in; trees in Figs. 1, S2). The discovery of subspecies *paui* to be conspecific to *C. laxula*, and its finding from the islands of Mallorca (Balearic Islands) and Elba (Tuscan archipelago), and thus Sicily, greatly expands the presence of this taxon in the Western Mediterranean, increasing its range into the Tyrrhenian shores (Fig. 5). There are no previous reports of this species for the Balearic and Elba Islands (see Pignatti, 1982; Innamorati, 1991; Bolòs & Vigo, 2001; Conti & al., 2005). This implies an important range expansion of *C. laxula* (=*C. sylvatica* subsp. *paui*) in Spain and a new taxon for the Italian flora.

Misidentifications of *C. laxula* and *C. sylvatica* may have been due to the very subtle morphological differences between both taxa (Table 3). Thus, the finding of more populations of *C. laxula* in other adjacent areas could be expected. In particular, given its presence in the Elba Island and Sicily, we recommend the study of additional mainland Italian populations. *Carex sylvatica* has been reported for the whole Italy (Pignatti, 1982), so it may be fairly probable that some Italian *C. sylvatica* populations, especially those occurring in Mediterranean habitats (see below), may indeed correspond to *C. laxula*. This same recommendation would be extensible to Corsica and SE France.

The misidentification of the Navarran populations of *C. sylvatica* subsp. *sylvatica* as *C. laxula* (=*C. sylvatica* subsp. *paui*; Luceño, 1994; Luceño & al., 2008) depicts a classical problem within taxonomy: those individuals of subsp. *sylvatica* displayed wider morphological variation than expected for the taxon (Global *Carex* Group, 2016).

The detailed examination of these materials reveals that they display characters, such as smooth upper side and margins of leaves, and the presence of only a few sparse prickles at the utricle beak, which do match those detected by *C. sylvatica* subsp. *sylvatica* in our study. Nevertheless, these specimens also displayed a higher number of masculine and androgynous spikes, as well as inflorescence length, than usually found in *C. laxula*.

4.3. Ecology

The ecology of *C. laxula* contrasts with the one reported for *C. sylvatica* subsp. *sylvatica* subspecies. The latter taxon inhabits Eurosiberian and Atlantic woods, being part of the understory in beech, oak and riparian forests, mostly on moist to wet soils on sandy or stony-clay substrates (Hegi, 1969; Luceño, 2008; Nilsson, 1985; Pignatti, 1982). In contrast, *C. laxula* mostly grows in shady humid Mediterranean forests dominated by *Quercus ilex* s.s., *Corylus avellana* woods, as well as riparian forests with *Alnus glutinosa* and *Populus* spp., mainly on siliceous substrates such as sandstones, at relatively low altitudes (150-300 m) (Maire, 1957; Luceño, 1994, 2008). The previous reports of *C. laxula* in beech forests (Luceño, 1994; Luceño & al., 2008) refer indeed to the Navarran populations here identified as *C. sylvatica* subsp. *sylvatica*, which is in agreement with the newly circumscribed ecology of both taxa.

4.5. Description

We provide an updated description for *Carex laxula* and an identification key to distinguish it from *C. sylvatica* subsp. *sylvatica*:

Carex laxula Tineo ex Boott, Carex 4: 202 (1867)
Lectotype (here designated): [ITALY] Sicily, Palermo, 1867, V. Tineo, s.n., BM001067082!

= *Carex algeriensis* Nelmes, Bull. Misc. Inform. Kew 1939: 99 (1939)
Lectotype (here designated) [ALGERIA], Yacoûren, between Bougie and Tizi Ouzou, ravine in deciduous oak forest, 27 April 1937, Alston and Simpson, 37614, (lectotype: K000363433, photo!; iso- BM000922723, photo!; BM000922724!, photo!).
= *Carex sylvatica* subsp. *algeriensis* (Nelmes) Maire & Weiller, Fl. Afrique N.4: 154 (1957)

= *Carex paui* Sennen, Exsicc. Pl. Espagne 1925: nº 5435 (1925)
Lectotype (designated by Luceño & al., 2008): [SPAIN] Barcelone, massif du Tibidabo, dans les barrancos, 6 June 1925, Fr. Sennen, Pl. Espagne 1925, n. 5435 (lectotype, MA 18049!; iso- MA 417028!). Icon.: Fig. 6.
= *C. sylvatica* subsp. *paui* (Sennen) A. Bolòs & O. Bolòs in Bolòs, Veg. Com. Barcelon.: 246 (1950)

Caespitose. Flowering stems up to 200 cm long, sharply trigonous, smooth. Inflorescences length 25–35 (70) cm. Leaves 10–14 mm wide, shorter than stems, ± carinate, ± rigid, with the adaxial surface scabrid; ligule 1.5-3(5) mm long, longer than wide, apex obtuse; basal sheaths pale brown, entire, rarely fibrous. Lowermost bract shorter than the inflorescence. Male spikes (1)2–4(7), 14–45 mm long, fusiform, sometimes with a few utricles at the base, very rarely with utricles also at the top. Female spikes 3–4, (22)30–53 mm long, occasionally shortly branched at the base, at least the lower ones separated from the upper ones, with long filiform and pendant peduncles, the upper ones with shorter peduncles, sometimes arising very close to each other. Androgynous spikes (0)1–2(4). Male glumes oblong to obovate, light brown, acute, subacute or obtuse, rarely mucronate; female glumes elliptic, shorter than the utricles, hyaline or, exceptionally, pale brown with a wide scarious margin. Utricles 4-4.5 × 1–1.2(1.5) mm, suberect, ovoid, trigonous, greenish or brownish, with only 2 prominent veins, more or less abruptly contracted into a narrow, slender, bifid beak, 1.2-2(2.3) mm long, conspicuously scabrid, with prickles towards the top. Achenes (2)2.2–2.5 × 0.9-1.4 mm, ovoid, trigonous, greenish to pale brown.

Distribution: Western Mediterranean endemic: NE Iberian Peninsula, Balearic Islands, Elba Island, Sicily, N Algeria, and N Tunisia [ALG BAL ITA SIC SPA TUN] (Fig. 5).
Notes: One of the isolectotype specimens we listed for *C. algeriensis* housed at BM (BM000922724) displays a label that says it was collected in May (5) instead of April, as indicated in the protologue and other specimens. However, the collector number (37614), and also the day of the month (27) and year (1937) is the same. We consider that the difference in the label is just a typo when transcribing the new label, thus the material should be considered an isolectotype.

4.6. Identification key

1. Male spikes 1(2); androgynous spikes absent or very rarely 1-2(3); utricle beaks smooth, very rarely with a few sparse prickles at the tip; leaves soft, smooth to slightly scabrid on the upper side and the margins; … *C. sylvatica* subsp. *sylvatica*

2. Male spikes (1)2-4(7); androgynous spikes 1-4, very rarely absent; utricle beaks conspicuously scabrid, with prickles towards the top; leaves ± rigid, conspicuously scabrid on most of the upper side … *C. laxula*

Table 1. Taxonomic treatment of *Carex* section *Sylvaticae* according to Egorova (1999), Jiménez-Mejías & Luceño (2011), Martín-Bravo & al. (2013), modified after the results of this paper and Global *Carex* Group (2016). Synonyms at species level have been taken from Govaerts & al. (2016). According to our results, *C. sylvatica* subsp. *paui* is already presented at species rank.

Accepted taxa	**Synonyms**	**Global distribution**
C. cretica Gradst & J. Kern		Crete
C. hypaneura V.I.Krecz.		S Caucasus
C. laxula Tineo ex Boott	*C. algeriensis* Nelmes, *C. paui* Sennen (=*C. sylvatica* subsp. *paui* (Sennen) A. Bolòs & O. Bolòs)	NE Iberian Peninsula, Balearic Islands, Tuscan archipelago, NE Algeria and N Tunisia, Sicily
C. rainbowii Luceño & *al*.		South Africa
C. sylvatica Huds.		
subsp. *sylvatica*		Europe to W Asia
subsp. *latifrons* (V.I.Krecz) Ö. Nilsson.	*C. latifolia* Boiss. & Balansa	NE Anatolia, Georgia and adjacent Caucasus (Nilsson, 1985)
C. vulcani Hochst. ex Seub.		Azores archipelago

Table 2. Variables included in the morphometric analysis reported as potential diagnostic characters. [1]The longest flowering stem is measured up to the beginning of the upper male spike. [2]Three measures considered (base, center, and beak). [3]Measures taken from three different utricles.

Continuous quantitative variable	**Description (measure)**
SW	Stem width (mm)
SL[1]	Stem length (cm)
LeafW	Leaf width (mm)
LeafL	Leaf length (cm)
LL	Ligule length (mm)
INFL[1]	Inflorescence length (cm)
BRINFLW	Inflorescence bract width (mm)
BRINFLL	Inflorescence bract length (cm)
MSW	Male spike width (mm)
MSL	Male spike length (mm)
FSW	Female spike width (mm)
FSL	Female spike length (mm)
MGW[2]	Male glume width (mm)
MGL[2]	Male glume length (mm)
MGBL[2]	Male glume beak length (mm)
FGW[2]	Female glume width (mm)
FGL[2]	Female glume length (mm)
FGHMW[2]	Female glume hyaline margin width (mm)
UW[3]	Utricle width (mm)
UL[3]	Utricle length (mm)
BULMW[3]	Base utricle length to maximum width (mm)
SUL[3]	Stigma utricle length (mm)
UBL[3]	Utricle beak length (mm)
ACHW[3]	Achene width (mm)
ACHL[3]	Achene length (mm)

SAL[3]	Stipe achene length (mm)
Discrete quantitative variable	
SPKMN	Male spikes number
SPKFN	Female spikes number
SPKAN	Androgynous spikes number
BUN	Beaks utricle number
NUN	Nerves utricle number
PBUN	Prickles beak utricle number
Qualitative variable	
PAP	Presence/absence papillae on the upper leaf

Table 3. Pairwise genetic distances calculated for species pairs in *Carex* sect. *Sylvaticae*. According to our results, *C. sylvatica* subsp. *paui* is here presented as *C. laxula*.

Species compared		**Genetic distance**		
		ITS	**5´trnK**	**Combined**
C. cretica	***C. rainbowii***	0.022	0.002	0.018
C. cretica	***C. laxula***	0.020	0.005	0.014
C. cretica	***C. sylvatica* subsp. *sylvatica***	0.004	0.005	0.006
C. rainbowii	***C. laxula***	0.039	0.003	0.014
C. rainbowii	***C. sylvatica* subsp. *sylvatica***	0.022	0.003	0.012
C. sylvatica* subsp. *sylvatica	***C. laxula***	0.016	0.007	0.009

Table 4. Main characters distinctive between *Carex sylvatica* subsp. *sylvatica* and *C. laxula* (=*C. sylvatica* subsp. *paui*); modified from Martín-Bravo & al. (2013) according to our own results.

	C. sylvatica **subsp. *sylvatica***	*C. laxula*
Longest flowering stem (cm)	100	200
Leaf upper side	Smooth to slightly rough	Strongly rough
Leaf width (mm)	(2)4–7(8)	(6)8–14
Male spikes number	1(2)	(1)2–4(7)
Female spike length (mm)	(23)25–55, not branched	(22)30–53, sometimes branched at the base
Utricle length (mm)	(3.8)4.0–5.0	(4.0)4.5–5.3
Utricle beak	Smooth, rarely with a few prickles towards the apex	Aculeolate

Table S1. Variables included in the analyses PCA (abbreviations specified in Table 2).

	Component		
Variable	**1**	**2**	**3**
SL	0.719	0.546	-0.309
INFL	0.736	0.560	-0.225
UL	0.706	-0.563	0.263
UBL	0.793	-0.446	0.333
SPKMN	-0.023	0.458	0.084
SPKAN	0.068	0.413	0.729
PAP	-0.176	0.404	0.623

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Appendix 1. Herbarium materials of *Carex sylvatica* subsp. *sylvatica* and *C. laxula* included in the morphological and molecular studies. Data between square brackets indicate the specimens also included in the molecular study, including sample labeling for the phylogeny, and ITS and 5'trnK Genbank number accession for the sequences newly generated in this study (if a marker is missing, it is replaced by a dash). Asterisks depict those samples included in the molecular study but not in the morphometric one. Herbaria acronyms are according to "Index Herbariorum" (Thiers, 2015).

Carex sylvatica* subsp. *sylvatica (materials previously classified as it)**:** ARMENIA**.** Lori, Pushkin pass, G. Fayvush *& al*., 03-0537 (MSB-123515). BOSNIA-HERZEGOVINA. Magli**,** P. Orendi, s.n. (M-0183079). BULGARIA. Central Balkans, P. Jiménez-Mejías & P. Vargas, 260PV04 (UPOS-4054), P. Jiménez-Mejías & F. Madroñal, 109PJM10 (UPOS-4049) [BUL4; KU242691, KU242704]*. CROATIA. Umag S., J. Höller, s.n. (M-0183078). DENMARK. **Odense.** H. F. Poulsen, s.n. (V-572147). FRANCE. **Atlantic Pyrenees**: Pau Urdos, Espelunguère, Les Forges d´Abel, P. Montserrat, s.n. (JACA 227895) [FRA2; KX426304, KX426309]; **Haute-Normandie:** Eure, P. Jiménez-Mejías 16PJM10 (UPOS-4112) [FRA1; -, KC122386]*. HUNGARY. **Budapest:** Ungvár, P. Erzberger, 3-1615 (B-100343844). GERMANY. **Baviera:** Münich, P. Jiménez-Mejías & G. E. Rodríguez-Palacios, 171PJM13 (UPOS-5559) [GER6; KU242692, KU242705]*. IRAN. **Tangerah:** Golestan National Park, H. Akhani, 10385 (M-0183092) [IRA1; KU242693, KU242706]. ITALY. **Abruzzo:** Central Apennines, Monti Della Laga National Park, P. Jiménez-Mejías *& al*., 246PJM10 (UPOS-4133) [ITA1; KU242694, KU242707]. **Piedmont:** colina di Turín, P. Jiménez-Mejías & E. Martinetto, 113PJM12-2 (UPOS-5350); ponte dei Preti P. Jiménez-Mejías & E. Martinetto, 63PJM12-2 (UPOS-5347). MONTENEGRO. **High Dinarics**: Durmitor National Park, Zabljak, P. Jiménez-Mejías, 228PJM10 (UPOS-4026) [YUG-MN1; KU242702, KU242716]. NORWAY. **Asker:** Konglungen, P. Jiménez-Mejías & K. Lye, 188PJM09 (UPOS-4547) [NOR1; -, KC122387]. POLAND. Upper Silesia, Rybnik, Krystof, 10362 (B-100118074). ROMANIA. Cotofanesti, D. Mititelu *& al*., s.n. (M-0183082). RUSSIA. **Kazan**, Semenenko & Nekrasova, s.n. (B-100448059). **Moscow**: Bei Dorf Weschke, A. K. Skvortsov, s.n. (M-0183087) [RUS5; (KU242700, KU242712]*. SERBIA. Carpatians, Djerdap, P. Jiménez-Mejías, 83PJM10 (UPOS-4204). SOUTH AFRICA. Western Cape, Knysna Diov, G. Lindeberg, s.n. (V-571678). SPAIN. **Jaén:** Siles, Las Acebeas, S. Martín-Bravo *& al*., 121SMB15 (UPOS-

6320) [SPA8; KX426307, KX426314]. **Girona:** Olot, La Moixina, P. Jiménez-Mejías *& al.*, 106PJM13 1/2 (UPOS-5270) [SPA2; -, KX426311]*. **Huesca:** Ansó, P. Montserrat, s.n. (JACA 80782) [SPA6; KX426305, KX426312], National Park Ordesa, M. L. Buide & J. M. Marin (UPOS-161) [SPA5; -, KU242714]*. **Lleida:** Les Bordes, Artiga de Lin, E. Maguilla & M. Luceño, 39EMS12 (10) 1/2 (UPOS-5048) [SPA1; -, KX426310]. Palencia: Piedrasluengas, J. M. Marín *& al.*, 14004JMM (UPOS-163) [SPA4; KU242699, -]*. SWEDEN. **Öland**: Högsrum, C. M. Norrman, s.n. (V-572178). **Västergötland:** Västra Tunhem, J. Sjögren, s.n. (V-572152) [SWE2; KU242701, KU242715]*.

C. sylvatica subsp. ***sylvatica*** (materials previously classified as *C. sylvatica* subsp. *paui*)**:** SPAIN. **Navarra:** Artikutza, I. Aizpuru & P. Catalán, s.n. (ARAN 22918); Foz de Arbayun, J & G. Montserrat, 87-JACA-0911-08102 (JACA 810287) [SPA9; KX426308, KX426315]; Garralda, G. & J. Montserrat, s.n. (JACA 797187); Isaba, L. Villar, s.n. (JACA 10045273) [SPA7; KX426306, KX426313]; Ochagavia, L. Villar & G. Monts, s.n. (JACA 118787).

***Carex laxula*:** ITALY. **Sicily:** 1877, V. Tineo, s.n. (BM-001067082; *C. laxula* lectotype). **Tuscany:** Elba, J. Höller, s.n. (M-0223069) [ITA3; KU242695, KU242708]; Elba, Mt. Perone, J. Höller, s.n. (M-0223070) [ITA4; KU242696, KU242709]. SPAIN. **Catalonia:** Barcelona: Massif du Tibidabo, F. Sennen, Pl. Espagne 1925, n. 5435 (*C. paui* lectotype, MA-18049, isolectotype, MA 417028; photos!); Montnegre, P. Jiménez-Mejías *& al.*, 85PJM13 1/13 (UPOS-6142) [SPA1; KU242697, KU242710]; Sant Carles, P. Jiménez-Mejías *& al.*, 76PJM13 1/4 (UPOS-6141) [SPA2; KU242698, KU242711]. **Mallorca:** Lluch, H. Merxmüller & W. Wiedmann, 7709 (M-0223072) [SPA3; -, KU242713]. TUNISIA. **Medjerda:** Bei Les Chênes, H. Hertel, 8305 (M-0183088) [TUN1; -, KU242703]*.

Appendix 2. Accession numbers for ITS and 5´trnK sequences downloaded from Genbank and included in the molecular study.

Carex* sect. *Spirostachyae*: *C. distans (EU483663, JN627754), ***C. punctata*** (DQ384180, EU812618); ***Carex* sect. *Ceratocystis*: *C. demissa*** (AY278307, JN627690), ***C. flava*** (AF285007, JN627705); ***Carex* sect. *Rhynchocystis*: *C. bequaertii***

(EU288572, KC122385), ***C. pendula*** (AY757600, KC122384); ***Carex* sect. *Sylvaticae*: *C. rainbowii*** 1 (KC122380, KC122382), ***C. rainbowii*** 2 (KC122381, KC122383), ***C. cretica*** 1 (DQ384117,-), ***C. cretica*** 2 (DQ384118, EU812677), ***C. sylvatica* subsp. *sylvatica*** GER5 (AY278306,-), ***C. sylvatica* subsp. *sylvatica* SWI2** (AY757599,-).

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Fig.1. Bayesian phylogenetic tree obtained from the concatenation of nrDNA ITS and ptDNA 5´trnK sequences for *Carex* section *Sylvaticae* (*C. cretica, C. laxula* (=*C. sylvatica* subsp. *paui*), *C. rainbowii*, and *C. sylvatica* subsp. *sylvatica*) and closely related sections (*Ceratocystis, Spirostachyae, Rhynchocystis*). Thirty-six samples were included in this analysis. Numbers above or below branches correspond to the posterior probability (PP>0.9, above branches) and Bootstrap (BS> 50%, below branches) support values. Arrows depict the samples previously classified as *C. sylvatica* subsp. *paui*.

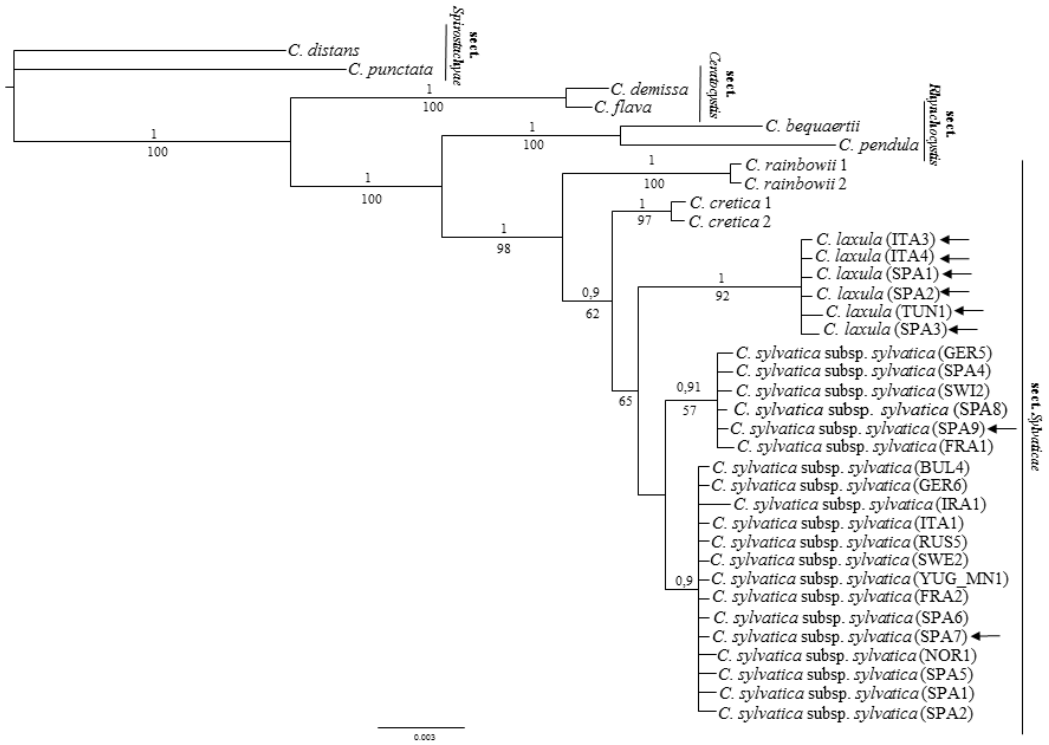

Fig.2. Scatter plot of first and second principal components (a) and the three first principal components extracted in the PCA analysis of the morphometric data. *Carex sylvatica* s.s. is represented by circles, *C. laxula* (=*C. sylvatica* subsp. *paui*) by triangles. Empty circles depict these individuals of *C. sylvatica* s.s. previously classified as subsp. *paui*

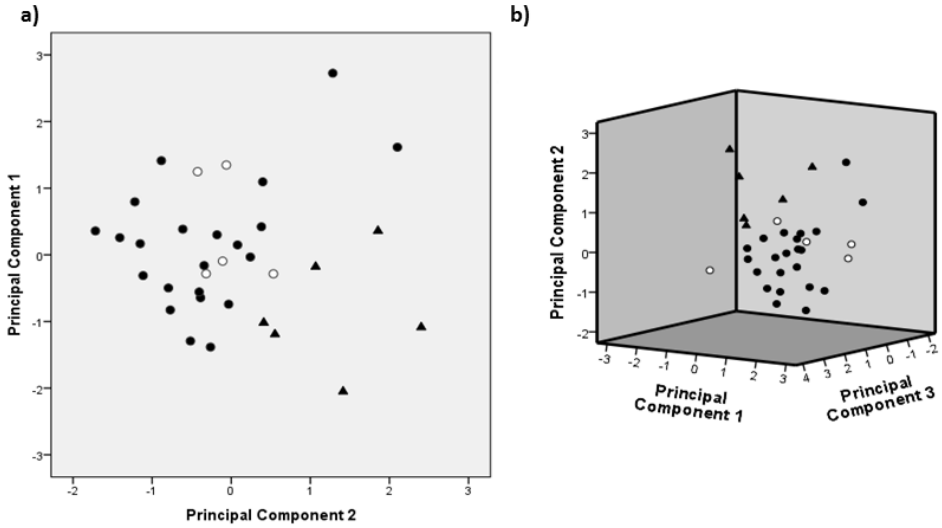

Fig.3. Boxplots of the most important diagnostic characters retrieved by PCA.

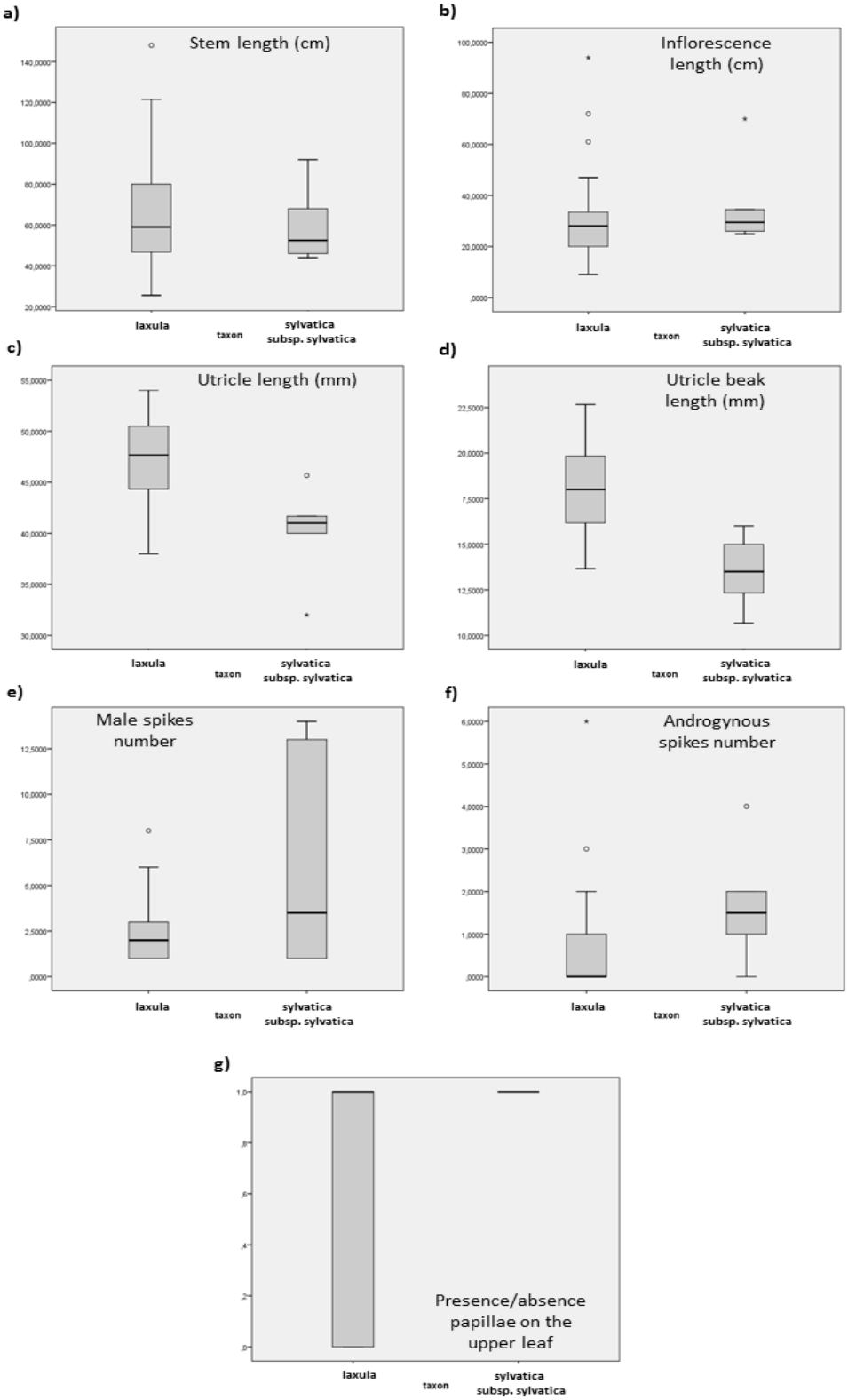

Fig.4. Detailed photographs of diagnostic characters distinguishing *Carex laxula* (= *C. sylvatica* subsp. *paui*; left column) and *C. sylvatica* subsp. *sylvatica* (right column). a, b) male spike(s) of the inflorescence; c, d) utricle beak and complete utricle (inset); e, f) leaf upper side. Scale bars: a, b=0.5 mm; c, d= 0.2 mm (0.1 mm in inset); e=0.2 mm; f=0.1 mm. Specimens used: a) J. Höller, s.n. (M-0223070); c, e) P. Jiménez-Mejías et al., 76PJM13 (UPOS-6141); b, d, f) M. Luceño et al., 1608ML (UPOS-3427).

Fig.5. Known distribution of *C. laxula* (=*Carex sylvatica* subsp. *paui*). Studied populations are represented by black triangles and literature records by white circles.

Fig.6. *Carex sylvatica* subsp. *paui.* a) Massif du Tibidabo a S. Medí, Barcelona, Spain (MA 18050); b-j) Argentona, Barcelona, Spain (MA 18494): a) basal part of a plant with lower half of a flowering stem; b) upper half of a flowering stem; c) ligule; d) male spike ; e) female spike ; f) male glume ; g) female glume ; h) utricle; i) cross section of a utricle; j) achene; k) cross section of an achene. Plate reproduced with permission from Flora Iberica (Luceño, 2008).

Fig. S.1. Bayesian phylogenetic tree obtained from the analysis of the nrDNA ITS sequences for *Carex* sect. *Sylvaticae* (*C. cretica*, *C. laxula* (=*C. sylvatica* subsp. *paui*), *C. rainbowii*, *C. sylvatica*) and closely related sections (*Ceratocystis*, *Spirostachyae*, *Rhynchocystis*). Twenty-nine samples were included in this analysis. Numbers above or below branches correspond to the posterior probability (PP>0.9, above branches) and Bootstrap (BS> 50%, below branches) support values.

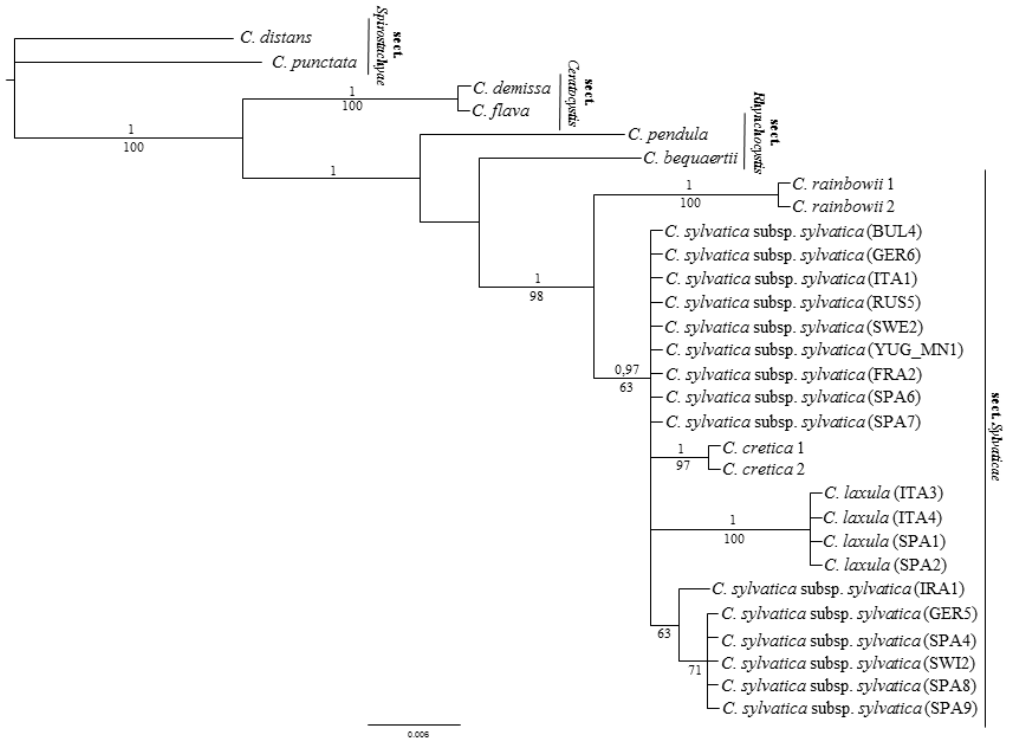

Fig. S. 2. Bayesian phylogenetic tree obtained from the analysis of the ptDNA 5´trnK sequences for *Carex* sect. *Sylvaticae* (*C. cretica*, *C. laxula* (=*C. sylvatica* subsp. *paui*), *C. rainbowii*, *C. sylvatica*) and closely related sections. (*Ceratocystis, Spirostachyae, Rhynchocystis*). Thirty-two samples were included in this analysis. Numbers above or below branches correspond to the posterior probability (PP>0.9, above branches) and Bootstrap (BS> 50%, below branches) support values.